PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 1–6 (1975)

Cyproheptadine and Beta Cell Function in the Rat: Insulin Secretion from Pancreas Segments *in vitro*[1] (38961)

DOUGLAS E. RICKERT[2] AND LAWRENCE J. FISCHER
(Introduced by H. E. Williamson)

The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa 52242

Cyproheptadine (CPH) causes alterations of structure and function in the rat pancreas (1, 2). Daily oral doses of CPH produce pancreatic β-cell vacuolization, altered glucose tolerance and nonfasting hyperglycemia. These abnormalities suggested that β-cells in CPH-treated rats may not be capable of normal insulin release. To investigate this possibility, glucose-stimulated and basal insulin release from pancreas segments obtained from CPH-treated rats were determined *in vitro*. Several stimulators of insulin release, which may act through different mechanisms, were employed to determine whether the CPH-damaged β-cell was generally refractory to such stimuli.

Both CPH and a metabolite desmethyl-CPH (DMCPH) are present in rat pancreatic tissue after a pancreotoxic dose of CPH (3). To examine whether the presence of these substances in the pancreas could alter insulin release, CPH and DMCPH were tested for their ability to modify glucose-stimulated insulin release from segments of pancreas taken from untreated rats.

The results of these studies help to explain the glucose intolerance which occurs in rats after administration of CPH and suggest that inhibition of insulin secretion due to treatment with this drug may be caused by more than one mechanism.

Materials and Methods. Male, Wistar rats (Simonsen Laboratories, Gilroy, Ca.) weighing 230–280 g were used. The animals had free access to food (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and water, and were housed in suspended, stainless steel cages under controlled lighting (lights off, 1900–0600 hr).

Cyproheptadine (4-(5H-dibenzo[a,d]cyclohepten-5-ylidine)-1-methylpiperidine) (CPH) was supplied as the hydrochloride monohydrate by the Merck Institute for Therapeutic Research, West Point, Pa. Desmethyl-CPH (DMCPH) was synthesized by the method of Engelhardt *et al.* (4).

Rats received CPH (45 mg/kg) by oral or ip administration in normal saline solution. The animals were sacrificed by decapitation at 3, 24, and 48 hr after a single dose of CPH and at the same times after the eighth daily dose of the drug. Control animals received normal saline (1.5 ml/100 g) in place of a CPH solution.

The experiments on insulin release were performed between 9 and 11 AM using the pancreas from nonfasted rats. The *in vitro* system for measuring insulin release was essentially that used by Feldman and Lebovitz (5). The pancreas was quickly removed and placed in oxygenated (95% O_2–5% CO_2) Krebs bicarbonate buffer (KBB) (6) at a glucose concentration of 3.3 m*M*. The fat and connective tissue were removed and the pancreas was transferred to fresh KBB. The KBB in this and all subsequent steps contained 100 KIU/ml Traysylol (FBA Pharmaceuticals, New York, NY) to retard proteolytic degradation of insulin in the medium during incubation (7). The pancreas was minced with scissors and washed by gentle shaking in an atmosphere of 95% O_2 and 5% CO_2 at 37° for 30 min. Approximately ten pancreatic segments weighing 2–4 mg each were then transferred to glass scintillation vials containing 2 ml of fresh KBB (3.3 m*M* glucose). The total wet weight of tissue in each vial ranged from 19 to 38 mg. The vials were incubated at 37° for 30 min under an atmosphere of 95% O_2–5% CO_2. At the end of this primary incubation period, the pieces were transferred to vials contain-

[1] This work was supported by USPHS-NIH Grant GM-12675.

[2] Present address: Department of Pharmacology, Michigan State University, East Lansing, Michigan.

ing 2 ml of KBB with glucose concentrations of 3.3 *or* 16.7 m*M*, and incubated for another 30-min period (test incubation). The media from the primary and test incubations were assayed for insulin.

Basal insulin release was determined by subtracting the amount of insulin released during the primary incubation (3.3 m*M* glucose) from that released during a test incubation at 3.3 m*M* glucose. Glucose-stimulated insulin secretion was determined by subtracting the amount of insulin released during the primary incubation (3.3 m*M* glucose) from that secreted during a test incubation at 16.7 m*M* glucose. A negative value for insulin release indicates that the amount of insulin secreted during the test incubation was less than insulin released during the primary incubation period.

The capabilities of secretogogues other than glucose to release insulin from the CPH-damaged pancreas were assessed by adding them to a test incubation containing 3.3 m*M* glucose. The wash and primary incubation also contained 3.3 m*M* glucose. Final concentrations during the test incubation were: 230 μg/ml L-leucine, 260 μg/ml dibutyryl 3′5′-cyclic AMP, 20 μg/ml glucagon, or 120 μg/ml tolbutamide. These concentrations were selected because they produced, at least a 250% increase in insulin release.

The effects of *in vitro* addition of CPH and DMCPH were studied in incubations of pancreas segments from control rats. The two compounds were added in aqueous solutions (0.1 ml) to yield final concentrations of 10^{-5} and 10^{-6} *M*. Only the test incubation contained CPH or DMCPH, thus allowing their effects on both basal insulin release and glucose-stimulated insulin secretion to be studied.

Immunoassay of insulin in aliquots (50 μl) of the incubation media was accomplished using a Sephadex bound antibody (Phadebas Insulin test, Pharmacia Laboratories, Piscataway, NJ). Insulin released from segments of rat pancreas is reported in immunological microunit equivalents of standard porcine insulin. All glassware which came into contact with insulin-containing solutions was silicon-coated with Siliclad (Clay-Adams, Parsippany, NJ) prior to use.

Statistical analyses were performed using an analysis of variance and the least significance difference test or Student's *t* test (8).

Results. The results in Table I show that glucose-stimulated insulin secretion was significantly depressed 3 and 24 hr after a single oral dose of CPH (45 mg/kg). Three hours after CPH administration insulin secretion in response to glucose was statistically indistinguishable from basal insulin release. Glucose-stimulated insulin secretion 24 hr after CPH was greater than basal insulin release, but it was significantly less than

TABLE I. INSULIN SECRETION FROM PANCREAS SEGMENTS OF RATS WHICH HAD RECEIVED A SINGLE ORAL OR INTRAPERITONEAL DOSE OF CPH[a]

	Hours after dose					
	3		24		48	
	Basal release	Glucose-stimulated secretion	Basal release	Glucose-stimulated secretion	Basal release	Glucose-stimulated secretion
Control (po)	1.5 ± 1.2	8.1 ± 1.6[b]	0.2 ± 0.9	9.5 ± 1.8[b]	−1.0 ± 1.5	6.8 ± 1.8[b]
CPH-treated (po)	0.5 ± 1.7	2.3 ± 1.9[c]	0.5 ± 0.9	4.0 ± 0.8[b,c]	−2.2 ± 1.2	9.4 ± 2.4[b]
Control (ip)	2.4 ± 0.9	5.7 ± 1.0[b]	0.2 ± 0.8	6.1 ± 1.5[b]	−1.4 ± 0.8	5.3 ± 0.9[b]
CPH-treated (ip)	1.2 ± 0.8	3.2 ± 0.9	1.5 ± 1.3	7.1 ± 2.4[b]	0.4 ± 0.4	7.3 ± 1.4[b]

[a] Dose of CPH was 45 mg/kg; controls received normal saline (1.5 ml/100 g). Values are μU insulin/mg wet wt pancreas/30 min. Each value is the mean ± SEM for four to five animals.

[b] Significantly different from respective basal insulin release (Student's *t* test, $P < 0.05$).

[c] Significantly different from respective control value (Student's *t* test, $P < 0.05$).

TABLE II. INSULIN SECRETION FROM PANCREAS SEGMENTS OF RATS TREATED FOR 8 DAYS WITH CPH[a]

	Hours after last dose					
	3		24		48	
	Basal release	Glucose-stimulated secretion	Basal release	Glucose-stimulated secretion	Basal release	Glucose-stimulated secretion
Control	0.3 ± 0.7	4.3 ± 0.7[b]	0.8 ± 1.0	9.5 ± 1.7[b]	2.8 ± 1.2	7.4 ± 1.0[b]
CPH-treated (po)	0.2 ± 0.2	1.0 ± 0.3[c]	0.3 ± 0.4	3.6 ± 1.4[c]	0.3 ± 0.3	6.1 ± 2.0[b]
CPH-treated (ip)	0.7 ± 0.3	1.4 ± 0.5[c]	0.9 ± 0.5	5.4 ± 1.6[b]	1.5 ± 0.7	6.5 ± 2.0[b]

[a] Dose of CPH was 45 mg/kg; controls received normal saline ip (1.5 ml/100 g). Values are μU insulin/mg wet wt pancreas/30 min. Each value is the mean ± SEM for four to five animals.

[b] Significantly different from respective basal insulin release (Student's *t* test, $P < 0.05$).

[c] Significantly different from respective control glucose-stimulated insulin secretion (Student's *t* test, $P < 0.05$).

control. When 48 hr separated CPH administration and measurement of insulin secretion, no drug effect was observed.

Since CPH-induced β-cell vacuolization is less severe after ip administration when compared to the oral route (1), insulin release was also assessed after a single ip dose of CPH (45 mg/kg). The results shown in Table I indicate that CPH given intraperitoneally caused less alteration in glucose-stimulated insulin release when compared to the inhibition caused by an identical oral dose of the drug. Only at 3 hr after an ip dose was glucose-stimulated insulin release not statistically larger than basal insulin release. This indicates a shorter duration for inhibition of hormone release after an ip dose of CPH since glucose-stimulated insulin release was still inhibited at 24 hr after an oral dose of the drug.

Morphologic alterations in the rat pancreatic β-cell caused by CPH become more pronounced as the treatment period is lengthened (2). In order to study insulin secretion at a time when marked vacuolization of β-cell cytoplasm had occurred, rats were given CPH (45 mg/kg) daily either orally or ip for 8 days prior to determination of *in vitro* insulin release. The results in Table II show that pancreas segments from rats sacrificed 3 hr after the eighth daily oral or ip dose of CPH were unable to respond to a glucose stimulus with insulin secretion above basal levels. Glucose-stimulated insulin secretion from pancreas segments of rats treated orally with CPH remained statistically indistinguishable from basal release at 24 hr after the eight daily dose. In contrast, pancreas segments obtained from ip treated rats at 24 hr after the eighth dose were able to respond to an elevated glucose concentration with insulin secretion greater than basal release. When 48 hr had elapsed between the eighth daily dose of CPH and measurement of *in vitro* insulin secretion, no drug effect was seen.

Glucagon, L-leucine, tolbutamide, and dibutyryl 3′5′-cyclic AMP were substituted for high glucose concentrations in order to determine whether CPH-treatment resulted in decreased insulin secretion in response to secretogogues other than glucose. The experiments were performed using pancreas obtained 24 hr after a single 45 mg/kg oral dose of CPH. The results in Table III show that CPH treatment reduced insulin secretion caused by various stimuli.

Preliminary experiments indicated that CPH added *in vitro* was capable of blocking glucose-stimulated insulin secretion from pancreas segments of control rats. In order to test whether this property was shared by its metabolite, CPH and DMCPH were tested for their ability to affect glucose-mediated insulin secretion *in vitro*. Table IV shows that both compounds significantly decreased glucose-stimulated insulin secretion at a concentration of 10^{-5} *M*. This effect was not observed at 10^{-6} *M* concentrations of CPH or DMCPH.

TABLE III. INSULIN SECRETION FROM PANCREAS SEGMENTS OF CONTROL AND CPH-TREATED RATS IN RESPONSE TO VARIOUS SECRETOGOGUES[a]

Treatment	Basal release	Leucine stimulated secretion	db.c-AMP stimulated secretion	Glucagon-stimulated secretion	Tolbutamide-stimulated secretion
Control	−1.0 ± 1.2	3.6 ± 1.5[b]	1.7 ± 0.3[b]	2.2 ± 0.4[b]	2.2 ± 0.8[b]
CPH	0.0 ± 0.4	1.3 ± 0.3[b, c]	0.1 ± 0.2[c]	−0.3 ± 0.7[c]	0.4 ± 0.4[c]

[a] CPH (45 mg/kg) or H_2O (1.5 ml/100 g) was given 24 hr prior to sacrifice. Both were given orally. Values are μU insulin/mg wet wt pancreas/30 min. Each value is the mean ± SEM for four to five animals.

[b] Significantly different from respective basal insulin release (analysis of variance, and least significant difference test).

[c] Significantly different from respective control value (analysis of variance and least significant difference test).

Discussion. The glucose intolerance (1, 2) and hyperglycemia (9) which develop in rats given CPH suggested that pancreatic β-cells were not capable of releasing insulin normally. The present study shows that treatment of rats with CPH causes an inhibition of glucose-induced insulin release. Glucagon and several other pharmacologic and physiologic stimuli were also unable to cause insulin secretion from pancreas segments of CPH-treated rats. In an earlier study, glucagon failed to elicit an increase in plasma insulin in rats given CPH for 14 days (1). These observations do not eliminate the possibility that peripheral insulin resistance could contribute to glucose intolerance in CPH-treated rats. However, the demonstrated inability of the β-cell to release normal amounts of insulin in response to stimuli adequately explains the lack of control for plasma glucose in rats given the drug.

It seems likely that the diminished insulin secretion seen 3 hr after CPH administration was due, at least in part, to the presence of CPH or DMCPH in the pancreas. Both compounds were shown to inhibit glucose-stimulated insulin secretion from normal pancreas at 10^{-5} *M* when added *in vitro* and both have been found in the pancreas at 3 hr after a dose of CPH (3) in concentrations exceeding that figure. Since neither CPH nor DMCPH can be detected in the pancreas 24 hr after CPH administration (3), the inhibition of insulin secretion at 24 hr after the last dose of CPH is not due to the presence of these compounds. Other metabolites of CPH do persist in the pancreas for at least 24 hr after administration of CPH (3). These unidentified substances could have an inhibitory action on insulin release.

TABLE IV. EFFECT OF *In Vitro* ADDITION OF CPH AND DMCPH ON INSULIN SECRETION FROM RAT PANCREAS SEGMENTS

Compound added	Concentration (*M*)	Basal release[a]	Glucose-stimulated secretion[a]
None (Control)	—	−0.4 ± 0.5	5.0 ± 1.0[b]
CPH	10^{-6}	−0.1 ± 0.4	3.5 ± 1.3[b]
	10^{-5}	−1.0 ± 2.5	−0.1 ± 1.0[c]
DMCPH	10^{-6}	0.6 ± 0.8	8.4 ± 2.8[b]
	10^{-5}	1.2 ± 0.4	0.4 ± 1.1[c]

[a] Each value is the mean ± SEM for four to five animals (control is mean ± SEM for nine animals). Values are μU insulin/mg wet wt pancreas/30 min.

[b] Significantly different from corresponding basal insulin release, $P < 0.05$, Student's *t* test.

[c] Significantly different from control (Student's *t* test, $P < 0.05$).

The inability of the pancreas to secrete insulin at 24 hr after the last dose of CPH may be due to a loss of pancreatic insulin. Longnecker *et al.* (2) observed that insulin secretory granules in the β-cell were fewer at 24 hr after an oral dose of CPH and Rickert *et al.* (9) showed that a significant reduction in the pancreatic content of insulin occurred at that time. The mechanism by which CPH reduces pancreatic insulin content is unknown. It is apparent from the

present study that the drug does not stimulate insulin release. On the contrary, release is inhibited. Since basal insulin release was not altered in pancreas segments taken from CPH-treated animals, the hormone is not leaking from damaged islets in detectable amounts. Studies are in progress to determine whether insulin synthesis, storage or degradation is altered in rats treated with CPH.

The importance of pancreatic insulin content in determining insulin release in CPH-treated rats is probably reflected in the difference between secretion seen 24 hr after an oral dose and that seen 24 hr after an ip dose. *In vitro* insulin secretion at that time was depressed after an oral dose of CPH, but it was normal after an ip dose of the drug. This may be due to the more marked decrease in pancreatic insulin after a 45 mg/kg oral dose of CPH than that observed after an identical ip dose of the drug (9). CPH-induced insulin depletion may also account for the fact that none of the secretogogues tested were able to elicit control levels of insulin secretion from pancreas segments taken from CPH-treated rats. Repletion of pancreatic insulin is nearly complete at 2 days following a 14-day course of CPH treatment (9). It seems reasonable to assume that repletion following the 8 days of treatment used in the present study would be at least as rapid. Therefore, the normal insulin secretion seen 48 hr after the last dose of CPH is not surprising, and may reflect a nearly normal pancreatic content of insulin.

Other recent reports have shown that CPH is an inhibitor of insulin release when added to *in vitro* systems. Because CPH and some antidepressants possessing the same tricyclic ring as CPH inhibited insulin release from the isolated perfused rat pancreas, Joost *et al.* (10) suggested that this effect was a common attribute of tricyclic compounds. Feldman *et al.* (11) showed that CPH inhibited monoamine oxidase in hamster pancreatic islets and suggested that this allowed endogenous amines to inhibit insulin release. It is doubtful that any of the suggested mechanisms for the inhibitory effect of CPH on insulin release are involved in the pancreotoxicity of the drug. This is because some other compounds having the same tricyclic structure as CPH do not cause β-cell vacuolization in the rat (12). In addition, hamsters do not exhibit CPH-induced β-cell toxicity (1) yet the drug inhibits insulin release in that species, presumably through an effect on monoamine oxidase. The known pharmacologic actions of CPH are apparently not involved in either its pancreotoxicity or its ability to inhibit insulin secretion. This reasoning arises from the fact that DMCPH is pancreotoxic (12) and inhibits insulin release as shown in this study but does not possess the antiserotonin or antihistaminic action of CPH (4).

Although the mechanisms by which CPH damages the rat pancreas are not clear, the present study shows that an inability of the pancreas to release insulin in response to an appropriate stimulus is a consequence of CPH administration. It appears that a direct inhibition of insulin secretion by the drug and its metabolite, as well as a drug-induced reduction of pancreatic insulin, may be involved in the functional deficiency in the rat pancreas caused by CPH.

Summary. Pancreatic islet cell vacuolization, hyperglycemia, and glucose intolerance develop in rats after oral administration of cyproheptadine (CPH). In order to determine whether these effects were associated with abnormal insulin secretion, pancreas segments from CPH-treated and control rats were compared for their ability to secrete insulin in response to several stimuli. Oral administration of CPH (45 mg/kg/day) to rats for 1 or 8 days inhibited glucose-mediated insulin secretion from pancreas segments obtained 3 and 24 hr after the last dose of the drug. Insulin secretion had returned to normal by 48 hr after drug administration. Intraperitoneal administration of the drug was less effective than oral administration in inhibiting *in vitro* insulin secretion. Other stimuli for insulin secretion (tolbutamide, glucagon, L-leucine, and dibutyryl 3′,5′cyclic AMP), like glucose, were incapable of releasing normal amounts of insulin from pancreas segments of CPH-treated rats.

CPH and a metabolite, desmethyl-CPH,

inhibited glucose-stimulated insulin secretion when added *in vitro* to pancreas segments from control rats. This suggests that the inhibition of insulin secretion in pancreas segments taken from animals treated with CPH could be due, at least in part, to the presence of drug and its metabolite in the tissue. A previously observed reduction in the pancreatic content of insulin in CPH-treated rats may also contribute to the abnormal insulin release in animals given the drug.

The authors thank Mr. William Raun and Mrs. Ann Grow for their excellent technical assistance.

1. Wold, J. S., Longnecker, D. S., and Fischer, L. J., Toxicol. Appl. Pharmacol. **19,** 188–201 (1971).
2. Longnecker, D. S., Wold, J. S., and Fischer, L. J., Diabetes **21,** 71–79 (1972).
3. Wold, J. S. and Fischer, L. J., J. Pharmacol. Exp. Therap. **183,** 188 (1972).
4. Engelhardt, E. L., Zell, H., Saari, S., Christy, M. E., Colton, D., Stone, C. A., Stavorski, J. M., Wenger, H. D., and Ludden, C. T., J. Med. Chem. **8,** 829 (1965).
5. Feldman, J. M. and Lebovitz, H. E., Endocrinology **86,** 66 (1970).
6. Krebs, H., Biochim. Biophys. Acta **4,** 249 (1950).
7. Burr, I. M., Stauffacher, W., Balant, L., Renold, A. E., and Grodsky, G., Lancet **1,** 882 (1969).
8. Steele, R. G. D. and Torrie, J. H., "Principles and Procedures of Statistics," p. 132. McGraw-Hill, New York (1960).
9. Rickert, D. E., Burke, J., and Fischer, L. J., J. Pharmacol. Exp. Therap. **193,** 585 (1975).
10. Joost, H. G., Poser, W., and Panten, U., Naun-Schmiedeberg's Arch. Pharmacol. **285,** 99 (1974).
11. Feldman, J. M., Chapman, B. A., and Plonk, J. W., Life Sci. **15,** 1121 (1974).
12. Fischer, L. J., Wold, J. S., and Rickert, D. E., Toxicol. Appl. Pharmacol. **26,** 288 (1973).

Received March 31, 1975. P.S.E.B.M., 1975, Vol. 150.