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CYPROHEPTADINE-INDUCED ALTERATIONS IN RAT INSULIN SYNTHESIS*

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Abstract—Experiments were conducted to determine whether the reduced pancreatic insulin content in rats treated with cyproheptadine is due to a drug-induced inhibition in insulin biosynthesis. Pancreatic islets were isolated six hours after the last of eight daily oral doses of cyproheptadine (45 mg/kg) or water (control). Islets from drug-treated animals were depleted of insulin and exhibited a significant hyperplasia. Addition of cyproheptadine *in vitro* to islets from drug-treated and control animals caused an inhibition of proinsulin synthesis, as measured by ^{3}H-leucine incorporation. The degree of inhibition was dependent on drug concentration and at 8×10^{-5} M the incorporation of label into proinsulin was completely prevented. The inhibitory effects of cyproheptadine were less pronounced when incorporation of label into total islet protein was measured. The drug was apparently less effective in altering the synthesis of other islet proteins and showed selectivity for inhibition of proinsulin synthesis. Measurement of proinsulin content in the whole pancreas of rats given cyproheptadine *in vivo* showed that a 77% reduction occurred by six hours after a single 45 mg/kg oral dose. The results indicate that an inhibition of proinsulin synthesis is responsible for the depletion of pancreatic insulin in rats given cyproheptadine.

Daily oral administration of cyproheptadine to rats has been shown to produce unique alterations in pancreatic beta cells [1]. Cyproheptadine treatment produces a vesiculation of the rough endoplasmic reticulum and a loss of insulin secretory granules within two days [2]. There is a concurrent depletion of pancreatic immunoreactive insulin and drug-treated animals become hyperglycemic [3]. With continued cyproheptadine treatment, the cytoplasmic vesicles in beta cells coalesce to form large vacuoles which can be observed by light microscopy after six to eight daily doses. The mechanism by which the drug produces these biochemical and morphologic alterations in the beta cell of the pancreas is not known but changes in insulin synthesis, storage, and secretion could be involved.

The present report describes experiments undertaken to investigate whether drug-induced alterations in proinsulin synthesis could be responsible for the depletion of pancreatic insulin caused by cyproheptadine treatment. The rates of proinsulin and insulin synthesis, as measured by ^{3}H-leucine incorporation, were determined in pancreatic islets isolated from rats treated with water or cyproheptadine and in islets subjected to drug exposure *in vitro*. It is apparent from the results of this study that cyproheptadine has the ability to markedly influence the synthesis of proinsulin.

MATERIALS AND METHODS

Animal treatment. Male Wistar rats (Simonsen, Gilroy, CA) weighing 180–220 g were given water, 15 ml/kg, or an aqueous solution of cyproheptadine (Merck Institute for Theurapeutic Research, West Point, PA) by gastric intubation, using a dose of 45 mg/kg. The drug or its vehicle was administered between 8:30 am and 9:30 am daily for 8 days. All animals were housed in suspended stainless steel, wire bottom cages and allowed free access to food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO) and tap water. The light cycle was controlled automatically with darkness from 6:00 pm to 6:00 am.

Effects of cyproheptadine on synthesis of rat proinsulin, insulin, and total islet protein. The synthesis of proinsulin and insulin, as measured by ^{3}H-leucine incorporation, was assessed in rat pancreatic islets isolated six hours after the eighth daily dose of cyproheptadine or water. Eight daily oral doses of the drug were used because previous studies showed that morphological and biochemical changes in beta cells were maximal using that treatment schedule [1–3]. Islets were isolated at six hours after the last dose because drug and metabolites in the pancreas are known to be in high concentrations at that time [4]. The procedure used for isolation of pancreatic islets was the collagenase method of Lacy and Kostianovsky [5]. Islets isolated from 3 to 4 rats receiving the same treatment (cyproheptadine or water) were pooled in a Kreb's bicarbonate buffer, pH 7.4, containing 3 mg/ml glucose, 0.2 mg/ml albumin and 13 essential amino acids as described by Eagle [6] including 0.05 mM of non-labeled leucine. This medium was equilibrated with 95% O_2/5% CO_2 prior to the addition of islets and maintained under this atmosphere during subsequent procedures. All glassware coming into contact with insulin or insulin producing tissue was coated with Siliclad (Clay–Adams, Parsippany, NJ).

Thirty islets were randomly selected from those obtained from a particular treatment group and transferred in 0.1 ml to a 2 ml smooth glass homogenizer tube. Another group of 30 islets was transferred

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from the same pool to a second homogenizer tube. These tubes served as incubation vessels. After a 10 min preincubation 37°, 50 μCi of ^{3}H-leucine (0.05 ml, Amersham/Searle, Arlington Heights, IL; sp. act. 54–59 Ci/mmole) was added to each tube and the incubation was continued for 40 min. The incorporation of ^{3}H-leucine in one of the tubes was stopped by the addition of 0.1 ml cycloheximide, 0.5 mg/ml (Sigma Chemical Co., St. Louis, MO). Incubation of the second tube was continued for another 120 min after the addition of 1000-fold excess of non-labeled leucine (0.1 ml). At the end of the 40 and 160 min incubation periods, samples were centrifuged, the media removed, and the islets washed 4 times with 0.1 ml of buffer medium containing non-labeled leucine, 10 mg/ml. Islets were then homogenized with a teflon pestle in 0.25 ml of acid–ethanol (2% conc. HCl). Bovine serum albumin and leucine (1 mg of each in 0.1 ml of distilled water) were added as carriers, and the tissues extracted for insulin using the procedure of Davoren [7]. The resulting extract was dissolved in 0.4 ml of 3 M acetic acid and the entire volume applied to Bio-Gel P30 columns, 0.9 cm × 50 cm, 100–200 mesh, to separate proinsulin and insulin [8]. The proteins were eluted with 3 M acetic acid at a rate of 0.06 to 0.08 ml/min. The elution volumes of proinsulin and insulin were determined in preliminary experiments using standard bovine proinsulin and rat insulin (Novo, Copenhagen, Denmark). One half milliliter of each eluate collected at 10 min intervals was mixed with a scintillation solution previously described [9], and the radioactivity determined. Quench corrections were made using an internal standard. The total amount of labeled proinsulin and insulin eluting from the chromatographic column was calculated. No corrections for the presence of ^{3}H-labeled C-peptide, which has the same elution volume as insulin, were made. Aliquots of the remaining 0.1 to 0.3 ml of each eluate from the column were used for detection of immunoreactive insulin.

In other experiments, the incorporation of ^{3}H-leucine into total islet protein was assessed. Islets from water and cyproheptadine-treated rats were isolated at 6 hr after the eighth daily dose. Triplicate samples containing 30 islets were incubated with 1 μCi of ^{3}H-leucine in the incubation medium described above. After incubation, islets were transferred to paper discs and prepared for the measurement of amino acid incorporation by the procedure of Mans and Novelli [10].

To determine the direct effect of cyproheptadine on proinsulin and insulin synthesis, ^{3}H-leucine incorporation was measured when cyproheptadine was added to islets obtained from cyproheptadine-treated and water-treated rats. The animals received cyproheptadine (45 mg/kg) or water for 8 days and islets were isolated 6 hr after the last dose, as previously described. Thirty islets in 0.1 ml of incubation medium were preincubated for 10 min with cyproheptadine. The drug was added to the islets in 0.01 ml of water to produce final concentrations of 1.6×10^{-6} M, 1.6×10^{-5} M and 8.0×10^{-5} M. After addition of 100 μCi (0.05 ml) of ^{3}H-leucine, a 40 min incorporation was performed. A duplicate sample was incubated for an identical period with labeled leucine and then allowed a 120 min chase period as described above. Cycloheximide was used to terminate incorporation and islets were analyzed for radiolabeled proinsulin and insulin. Incorporation of ^{3}H-leucine into total protein was measured in islets from water and drug-treated rats in the presence of cyproheptadine at concentrations of 1.6×10^{-5} M and 8.0×10^{-5} M. The analysis for ^{3}H-labeled total protein was performed as previously described.

Alterations in pancreatic proinsulin and insulin in vivo. To determine the effect of cyproheptadine treatment on the content of proinsulin and insulin in the rat pancreas, the following study was performed. Rats were administered water or cyproheptadine (45 mg/kg/day) for 1 or 2 days and were sacrificed at 6 and 24 hr after the last dose. The pancreata were removed, homogenized in water (2.8 ml/g tissue) and extracted by acid–ethanol according to the procedure of Davoren [7]. The resulting extract was fractionated on Bio-Gel P30 columns to separate proinsulin and insulin [8]. The eluates containing proinsulin were pooled and assayed for insulin immunoreactivity (see below). Insulin containing fractions were handled in an analogous manner.

Plasma glucose, pancreatic islet insulin, protein and DNA assays. Blood was collected by cardiac puncture from water and cyproheptadine-treated rats at 6 hr after the eighth daily dose, mixed with heparin and plasma obtained by centrifugation. Plasma glucose concentrations were measured using the glucose oxidase method of Thompson [11]. The insulin content of acid–ethanol extracts of 30 islets isolated from rats treated with water or cyproheptadine for 8 days was determined by radioimmunoassay. The assay employed a rat insulin (Novo Research Institute, Bagsvaerd, Denmark) standard and the alcohol precipitation method of Makulu *et al.* [12]. Guinea pig anti-insulin serum and ^{125}I-insulin were purchased from Dr. P. H. Wright (University of Indiana, Indianapolis, IN) and New England Nuclear (Boston, MA; sp. act. ~ 100 μCi/mg), respectively. Serial dilutions of pancreatic extracts from water and cyproheptadine-treated rats gave identical curves to that of standard rat insulin. This indicated that the immunoreactivity of insulin extracts from drug-treated and control animals was not altered. Pancreatic islet DNA and protein content were determined using 100 islets isolated from water or cyproheptadine-treated rats at 6 hr after the eighth dose. Isolated islets were dissolved in 0.5 ml of 0.3 N NaOH (1 hr at 37°) and aliquots were taken for DNA and protein determinations. The DNA and protein assays were those of Kissane and Robbins [13] and Lowry *et al.* [14], respectively. The assays employed bovine serum albumin (Sigma Chemical Co., St. Louis, MO) and calf thymus DNA (Sigma Chemical Co., St. Louis, MO) as standards.

The size of pancreatic islets was assessed by randomly selecting islets isolated from water and cyproheptadine-treated rats and measuring their dimensions using a dissecting microscope equipped with a reticle scale. The method used to calculate the areas of isolated islets has been previously described [15].

Electron microscopy. Approx. 100 islets, isolated 6 hr after the eighth dose of water or cyproheptadine, were obtained for electron microscopy. The islets

were transferred to BEEM capsules and collected as a pellet by centrifugation. Fixation, dehydration and embedding, as described previously [2] were performed in these capsules. Sections were examined, using electron microscopy, for drug-induced alterations in beta cell ultrastructure.

Statistics. Data were analyzed by grouped student *t*-test or randomized complete block analysis of variance [16]. In the latter analysis, means were compared by the procedure of Dunnet [16]. The confidence limit in all analyses was 95%.

RESULTS

Characterization of cyproheptadine-induced changes in isolated islets. The beta cells in islets isolated from cyproheptadine-treated rats were examined to determine whether ultrastructural and biochemical changes, characteristic of cells in the intact pancreas of drug-treated animals, were present in the isolated cells. Fig. 1 shows a representative electron micrograph of beta cells from islets isolated from water-treated rats. The rough endoplasmic reticulum, mitochondria, and nuclei were normal in appearance and numerous insulin secretory granules were present. Islets isolated from cyproheptadine-treated rats exhibited marked degranulation, vesiculation of the rough endoplasmic reticulum and large cytoplasmic vacuoles (Fig. 1). Alpha cells were normal in appearance, exemplifying the cell specific changes produced by cyproheptadine treatment.

The immunoreactive insulin content of isolated islets was measured to further characterize the drug-induced beta cell damage. The results in Table 1 show that islets isolated from cyproheptadine-treated rats were almost completely depleted of insulin. The 93% decrease in insulin content in islets from drug-treated rats is consistent with the loss of secretory granules observed with the electron microscope (Fig. 1). A moderate hyperglycemia was also observed in drug-treated rats from which islets were obtained for insulin measurements. Water and cyproheptadine-treated animals had plasma glucose concentrations of 136 ± 5 mg/100 ml (mean $\pm$ S.E., N = 24) and 349 ± 30 mg/100 ml (mean $\pm$ S.E., N = 25), respectively.

Possible alterations in pancreatic islet size due to cyproheptadine treatment were investigated. Cyproheptadine treatment produced a 50% increase in islet size (Table 1). A corresponding increase in islet protein and DNA was observed following cyproheptadine treatment (Table 1). The magnitudes of the increases in islet DNA and islet protein were similar suggesting that the increase in islet size was due to hyperplasia.

Proinsulin, insulin, and total protein synthesis in pancreatic islets. The rates of incorporation of label from ^{3}H-leucine into proinsulin and insulin in islets isolated from drug-treated and water-treated rats were determined to detect possible changes in insulin synthesis due to cyproheptadine administration. Preliminary experiments showed that incorporation of label into proinsulin was linear with time for 60 min. The formation of proinsulin was routinely examined in islets incubated for 40 min with ^{3}H-leucine while the conversion of proinsulin to insulin was studied using a 40 min incorporation with ^{3}H-leucine followed by a 120 min chase with non-labeled leucine. Results

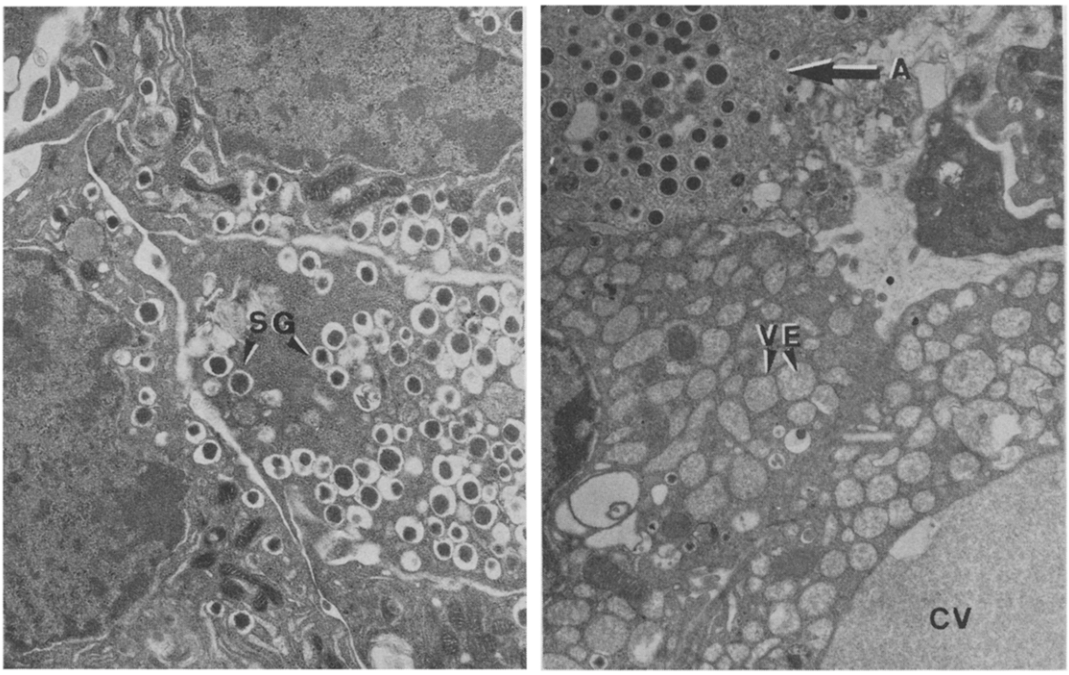

Fig. 1. Electron micrographs of islets isolated from water-treated (left panel) and cyproheptadine-treated (right panel) rats. All cells are beta cells except for the indicated alpha cell. Animals received water, 15 ml/kg, or cyproheptadine, 45 mg/kg, for 8 days and islets were obtained 6 hr after the last oral dose. A = alpha cell; CV = cytoplasmic vacuole; VE = vesicle; SG = secretion granule. Magnification = 11,400.

Table 1. Effect of cyproheptadine treatment on pancreatic islet size and on content of insulin, DNA, and protein*

Treatment	Immunoreactive insulin (ng/islet)	Area (reticle units²)†	Protein (ng/islet)	DNA (ng/islet)
Water (control)	49.6 ± 1.9	0.08 ± 0.00	517 ± 60	18.7 ± 1.8
Cyproheptadine	3.4 ± 0.7‡	0.12 ± 0.01‡	866 ± 69‡	24.9 ± 1.7‡
Cyproheptadine (% control)	7	150	200	140

* Islets were isolated 6 hr after the last of eight daily oral doses of cyproheptadine (45 mg/kg) or water. Value are mean ± S.E. for 8 experiments (insulin values are from 4 experiments).

† 1 reticle = 0.6 mm.

‡ Significantly different from water-treated control by student *t*-test, $P < 0.05$.

from a representative experiment showing the separation of insulin and proinsulin by exclusion chromatography are shown in Fig. 2. These data document that an adequate separation of the prohormone and insulin was achieved and show that the chase period results in conversion of proinsulin to insulin.

The results in Fig. 3 show that islets isolated from cyproheptadine-treated rats incorporated 740 per cent more radioactivity into proinsulin when compared to islets from animals receiving water. Labeling of insulin during 40 min was also enchanced in islets isolated from drug-treated animals. Since the islets from rats receiving cyproheptadine were 50 per cent larger than those from water-treated animals, the data could also be expressed as fmoles of ^{3}H-leucine incorporated per μg DNA using the previously determined (Table 1) appropriate values for islet DNA. After correction for the difference in islet size, the increases in incorporation of radiolabel into proinsulin and insulin in islets from drug-treated animals were still 400–500 per cent. The conversion of ^{3}H-labeled proinsulin to insulin was apparent in islets from drug-treated and control animals when a 40 min incorporation period was followed by a 120 min chase with non-labeled leucine (Fig. 3). More radiolabel was associated with insulin than proinsulin after a 120 min chase period indicating that conversion from proinsulin was occurring in the isolated islets. The data in Fig. 3 also show that islets isolated from cyproheptadine-treated animals contained more labeled insulin after the chase period when compared to islets from water-treated animals.

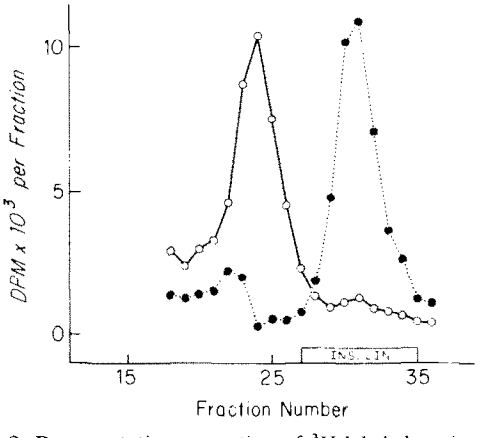

Fig. 2. Representative separation of ^{3}H-labeled proinsulin and insulin on Bio-Gel P30 columns. Islets were obtained from cyproheptadine-treated rats and incubated with ^{3}H-leucine. Following the incubation, acid ethanol extractable proteins were separated by gel filtration and the radioactivity per fraction was determined. Results from a 40 min incorporation with ^{3}H-leucine are shown (O——O) along with those obtained using a 40 min incorporation period followed by a 120 min chase with excess non-labeled leucine (●——●). The bar indicates the elution vol. of immunoreactive insulin; proinsulin elutes in fractions 20–26.

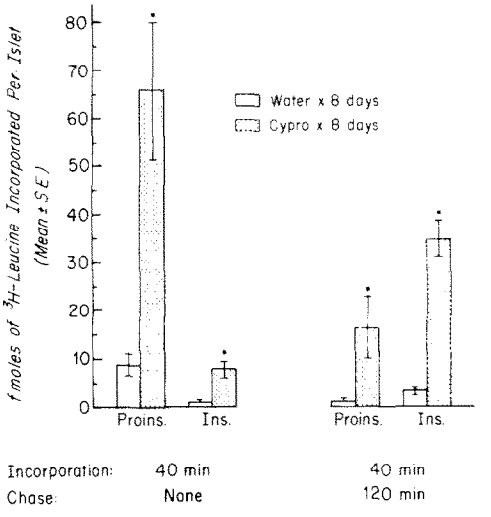

Fig. 3. Synthesis of proinsulin and insulin (^{3}H-leucine incorporation) in pancreatic islets isolated from water-treated and cyproheptadine-treated rats. Labeled proinsulin and insulin were measured following incubation of islets with ^{3}H-leucine using a 40 min incorporation period and after a similar incorporation period followed by a 120 min chase. The results represent 5–7 separate experiments. Asterisks indicate a significant difference from results in water-treated (control) animals.

Experiments were conducted to determine whether the increase in proinsulin synthesis occurring in islets isolated from cyproheptadine-treated rats was accompanied by a similar increase in the synthesis of total islet proteins. In these studies, islets were isolated from rats treated with water or cyproheptadine and the incorporation of ^{3}H-leucine into total islet protein was measured after 40 min. Islets from water-treated animals incorporated 1242 ± 208 (mean ± S.E., N = 5) fmoles of labeled amino acid per islet while islets from drug-treated animals showed incorporation of 2638 ± 688 (mean ± S.E., N = 5) fmoles per islet. The increase in synthesis of total islet proteins in islets from drug-treated animals was about 100% and there-

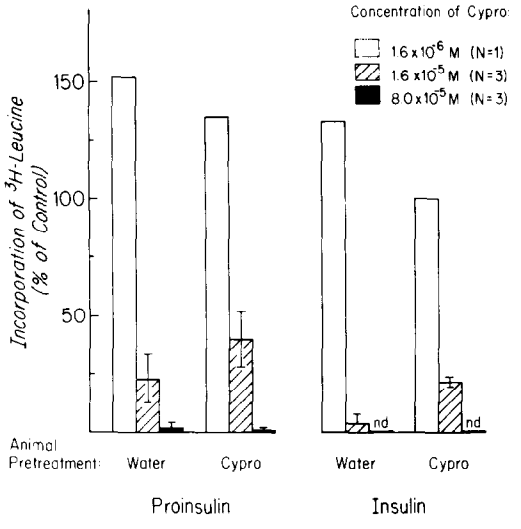

Fig. 4. Inhibition of proinsulin and insulin synthesis by addition of cycloheptadine to pancreatic islets isolated from water and drug-treated (CYPRO) rats. Labeled proinsulin was determined using a 40 min incorporation with ^{3}H-leucine and labeled insulin was assessed after a 120 min chase period. Incorporation of label in the presence of various concentrations of cycloheptadine was compared to a control sample containing no added drug. Results are expressed as the percent of the incorporation observed in the control sample. Bars denote mean ± S.E., "nd" indicates no detectable incorporation.

fore, not as large as the 700 per cent increase observed in the synthesis of proinsulin. This suggested that proinsulin synthesis was increased to a larger extent in islets from cycloheptadine-treated rats than the synthesis of other islet proteins.

Direct effects of cycloheptadine on synthesis of proinsulin, insulin and total islet proteins. The numerous washes of tissue associated with the collagenase isolation procedure may remove cycloheptadine from pancreatic islets obtained from drug-treated animals. The drug, therefore, was added to the incorporation medium and proinsulin and total protein synthesis was assessed in islets obtained from water and cycloheptadine-treated animals. The results in Fig. 4 show that, at a cycloheptadine concentration of 8×10^{-5} M, incorporation of radioactive amino acid into proinsulin and insulin was virtually non-existent in islets obtained from both water and cycloheptadine-treated animals. The incorporation of label into proinsulin and insulin was markedly depressed by the addition of 1.6×10^{-5} M cycloheptadine to the islets from each source. In a single experiment using a lower concentration of 1.6×10^{-6} M, cycloheptadine caused no apparent reduction of the labeling of proinsulin and insulin.

The effect of various concentrations of cycloheptadine on ^{3}H-leucine incorporation into total islet protein was investigated to determine whether the drug possibly altered synthesis of other pancreatic islet proteins. Islets obtained from animals treated with cycloheptadine or water were also used in these experiments and the results are shown in Fig. 5. At a concentration of 1.6×10^{-5} M, cycloheptadine had no significant effect on total protein synthesis in isolated islets. At a 5-fold higher concentration the drug produced a 60 per cent decrease in the incorporation of ^{3}H-leucine-derived radioactivity into islet protein. Comparison of these results with the direct effects of cycloheptadine on proinsulin synthesis indicates the drug apparently has selective inhibitory effects on the synthesis of proinsulin. At the highest concentration used, 8×10^{-5} M, cycloheptadine produced a complete inhibition in the synthesis of proinsulin and had a much smaller effect on the synthesis of total islet proteins. Similarly, 1.6×10^{-5} cycloheptadine was more inhibitory to proinsulin synthesis than to the synthesis of total islet protein.

Pancreatic proinsulin and insulin content in cycloheptadine-treated rats. To determine whether cycloheptadine may inhibit proinsulin synthesis *in vivo*, the pancreatic content of the prohormone and insulin was assessed in animals given the drug. A 77% decrease in pancreatic proinsulin was observed 6 hr after a single dose of cycloheptadine while insulin levels at that time remained unaltered (Fig. 6). At 24 hr after a dose of cycloheptadine, proinsulin levels were still significantly depressed and the insulin content of the pancreas was decreased by 84 per cent. Six hr after 2 doses of cycloheptadine both proinsulin and insulin were depressed and 18 hr later the reduction was still apparent. Thus, the administration of cycloheptadine to rats results initially in a decrease in pancreatic proinsulin followed by a similar decrease in insulin.

DISCUSSION

Inhibition of proinsulin synthesis by cycloheptadine. Addition of cycloheptadine to incubation media containing islets isolated from drug-treated or water-treated (control) animals caused a marked decrease in proinsulin synthesis. The concentrations used here to inhibit proinsulin synthesis are similar to concentrations of cycloheptadine and its metabolites in the

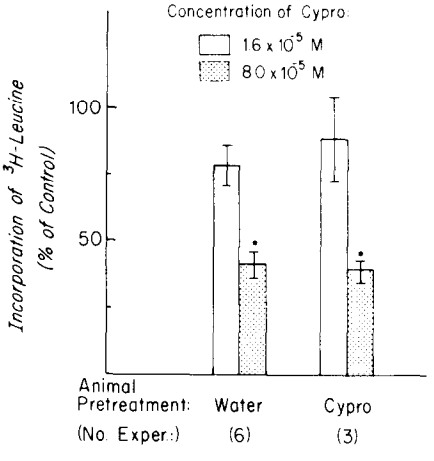

Fig. 5. Inhibition of total protein synthesis by addition of cycloheptadine to pancreatic islets isolated from water-treated and drug-treated (CYPRO) rats. A 40 min incorporation with ^{3}H-leucine in the presence of added cycloheptadine was utilized. A control sample contained no added drug, and incorporation results are expressed as percent of that occurring in the control. Bars denote the mean ± S.E. An asterisk denotes a statistical difference ($P < 0.05$) from control.

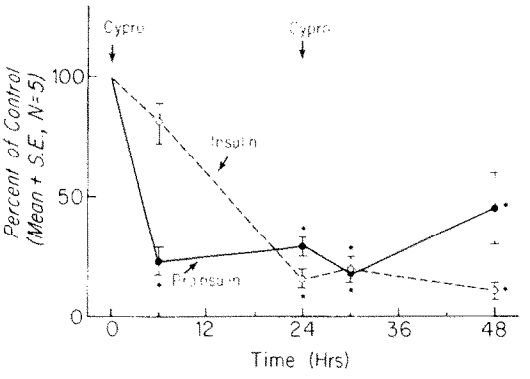

Fig. 6. Reduction of pancreatic proinsulin and insulin content by cyproheptadine *in vivo*. Rats were given cyproheptadine (CYPRO, ↓) 45 mg/kg, or water 15 ml/kg, daily for 2 days, and pancreatic insulin and proinsulin were determined at 6 and 24 hr after each dose. Results are expressed as percent of the proinsulin and insulin content in control animals receiving water (mean ± S.E., N = 4). Asterisks denote a statistical significance from respective water-treated controls using a group *t* test. Mean control values (ng IRI/mg) for proinsulin and insulin at the 6 hr time period of 2.1 ± 0.1 and 40.3 ± 7.2 were not significantly different from the respective control values at other time periods.

rat pancreas after a dose of the drug [4]. The ability of cyproheptadine to block the biosynthesis of proinsulin *in vitro*, as demonstrated here, could reasonably result in the depletion of insulin previously observed in rats treated with the drug [3].

In the present study, data were obtained to indicate that cyproheptadine treatment may indeed alter proinsulin synthesis *in vivo*. The depression of pancreatic proinsulin levels after one or two doses of the drug is consistent with the inhibition of synthesis of this prohormone observed *in vitro*. Since the intracellular pool of proinsulin is small [17] and assuming cyproheptadine has no effect on conversion of the prohormone to insulin, pancreatic proinsulin would be quickly depleted in the absence of its synthesis. Without a precursor available, insulin stores would also be decreased but at a later time. Data reported here indicates that the depletion of proinsulin and insulin follow this sequence after administration of the drug. Indirect evidence that cyproheptadine can also inhibit insulin formation in islet cells undergoing tisse culture is available. Richardson *et al.* [18] observed a decrease in basal insulin secretion and a loss of insulin secretion granules from beta cells when 5×10^{-4} M cyproheptadine was added to culture medium containing rat pancreatic islets. According to our results, that concentration would completely block proinsulin synthesis.

The inhibitory effect of cyproheptadine on islet-cell protein synthesis appeared to exhibit selectivity for proinsulin. Evidence for a selective inhibition of proinsulin synthesis by drugs has appeared in only a few reports. Levy and Malaisse [19] showed that diazoxide (0.2 mg/ml) and some sulfonylureas including tolbutamide (0.2 mg/ml) could produce a moderate inhibition of ^{3}H-leucine incorporation into proinsulin while having little effect on synthesis of other islet proteins. This effect was more pronounced at low concentrations (1.0 mg/ml) of glucose than at 3 mg/ml glucose. Cyproheptadine is apparently a more potent inhibitor of proinsulin synthesis than tolbutamide since, under similar conditions, it produced a greater inhibition at a concentration 1/50th that of the sulfonylurea. Comparison of our results with those obtained with cycloheximide indicates that cyproheptadine is as potent as the well known inhibitor of protein synthesis in its ability to reduce proinsulin formation in isolated islets [20]. Gunnarsson [21] reported a selective decrease in insulin synthesis in mouse islets isolated 10 min after diabetogenic doses of alloxan and streptozotocin. However in that study synthesis of insulin and other islet proteins was inhibited 100 and 75 per cent respectively, suggesting that the decrease in synthetic activity was not particularly specific for proinsulin. Recently Maldanato *et al.* [22] showed that streptozotocin inhibition of proinsulin synthesis was slightly greater than its inhibitory effects on synthesis of total islet protein. They concluded, however, that this probably did not represent a specific effect for proinsulin because of the unaffected synthesis of total proteins in islet cells other than beta cells. When a similar analysis is made using the data reported here, it can be concluded that cyproheptadine, unlike streptozotocin, exhibits specificity for inhibition of proinsulin synthesis.

Proinsulin and insulin synthesis in pancreatic islets depleted of insulin. The greatly enhanced incorporation of ^{3}H-leucine into proinsulin in islets isolated from cyproheptadine-treated rats is consistent with the known ability of beta cells to rapidly recover from the reduction in pancreatic insulin caused by cyproheptadine administration. Regranulation of beta cells and the return of pancreatic insulin levels to above normal values has been shown to occur *in vivo* within 48 hr of drug withdrawal [3]. Removal of the drug from the body due to metabolism [4] is apparently associated with a resumption in the *in vivo* synthesis of proinsulin. The islet isolation procedure used in the present study involves numerous washes to eliminate proteolytic enzyme activity and it is possible that cyproheptadine, or an inhibitory metabolite was removed during the preparation of the islets. It seems unlikely that inhibitory concentrations of cyproheptadine remain in islets isolated from drug-treated animals. If cyproheptadine or inhibitory metabolites remained in the islets, the addition of cyproheptadine *in vitro* would be expected to cause a greater degree of inhibition than that seen from addition of the drug to islets from water-treated animals. This was not observed. In addition, evidence exists from *in vitro* experiments which shows that ^{14}C-cyproheptadine can be removed from islets by washing the tissue in albumin-containing buffer (unpublished results).

Two explanations can be offered for the increased synthesis of proinsulin in pancreatic islets from which cyproheptadine has been removed. The first involves the ability of glucose to stimulate proinsulin synthesis at the transcriptional level [23]. Isolated islets from rats which have previously received infusions of glucose are known to synthesize proinsulin at a much higher rate than islets from normoglycemic animals [24]. Since hyperglycemia exists in cyproheptadine-treated rats [3], the synthesis of proinsulin in isolated islets from these animals could be increased by the mechanisms involved in glucose stimulation. Another

possible explanation for increased synthesis involves the fact that cyproheptadine treatment causes a depletion of insulin in the beta cells. An internal feedback mechanism may exist in those cells which, upon detection of insulin loss, stimulates synthesis of the hormone. Such a feedback mechanism may not have been unmasked until now because agents which reversibly reduce pancreatic insulin content without directly stimulating synthesis have not previously been studied in this way.

Cyproheptadine-induced changes in pancreatic islets. Pancreatic islets isolated from cyproheptadine-treated rats exhibited morphologic and biochemical changes identical to those previously observed in the intact pancreas of treated animals [2, 3]. Richardson [25], in assessing the histological changes produced by cyproheptadine in the rat pancreas, noted a 30% increase in the size of islets is animals receiving the drug. Data in the present study support those results and, in addition, show that the enlarged size is apparently due to hyperplasia of islet cells.

The hyperplasia of islet cells in rats receiving cyproheptadine could result from the hyperglycemic state of these animals. Mouse islets have been shown to respond to elevated glucose with an increase in DNA synthesis [26, 27]. Brotsky *et al.* induced hyperglycemia in rats by glucose infusion and observed a 14-fold increase in mitotic divisions of islet cells and a 2-fold increase in the volume of the islets [28]. In light of the beta cell alterations in rats administered cyproheptadine, the increase in islet size could be a result of proliferation of beta cells as they attempt to increase their insulin secreting capabilities in response to a high glucose environment and possibly a loss of insulin stores.

Some aspects of the mechanism by which cyproheptadine produces alterations in rat pancreatic beta cells are elucidated by the present study. The data suggest that the depletion of pancreatic insulin caused by cyproheptadine is the result of a drug-induced inhibition of proinsulin synthesis. Several possible mechanisms for this inhibitory effect of the drug are under investigation. The relationship between the drug-induced inhibition of proinsulin synthesis and the eventual formation of large cytoplasmic vacuoles in pancreatic beta cells of drug-treated animals is not known. Of particular importance, however, is a recent report which showed that addition of cyproheptadine to rat pancreatic islets in tissue culture caused ultrastructural changes in beta cells identical to those resulting from drug treatment *in vivo* [18]. The concentration of cyproheptadine in the culture medium was ten times greater than that used here to produce complete inhibition of proinsulin synthesis. This suggests that cyproheptadine inhibition of proinsulin synthesis leads not only to loss of insulin from beta cells but possibly to vesiculation of the rough endoplasmic reticulum and the formation of large cytoplasmic vacuoles.

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