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SHORT COMMUNICATIONS

Failure of Cyproheptadine to Induce Pancreatic B-Cell Lesion in Hypophysectomized Rats

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Summary. 40 mg/kg/day of cyproheptadine was given to groups of sham-operated and hypophysectomized young adult male rats by stomach tube for 10 days. Sham-operated and hypophysectomized control groups received vehicle only. In the sham-operated group cyproheptadine produced typical pancreatic B-cell vacuolization and degranulation, visible on light microscopy. Ultrastructural changes were mainly restricted to the rough endoplasmic reticulum where cysternal dilation, vesicle fusion and vacuole formation were common. The mean diameter of the islets of Langerhans was increased by 30%. Resting blood glucose levels were elevated significantly. No such findings were observed in the treated, hypophysectomized group of rats, indicating the importance of an intact pituitary for the genesis of these lesions and the accompanying hyperglycemia. The results also demonstrate that the effect of cyproheptadine on rat B-cells is not simply direct. The hypothesis that cyproheptadine may produce pancreatic B-cell changes through a drug-induced stimulation of pituitary or hypothalamic function is discussed.

Key words: B-cell vacuolization, cyproheptadine, hypophysectomized rats, pancreatic islet hypertrophy, hyperglycemia, drug-induced pancreatic B-cell lesions.

Cyproheptadine (Periactin® Merck Ltd.), a potent antiserotonin-antihistaminic compound with a structure similar to the tricyclic antidepressants [1], has been shown to stimulate appetite in man [2] and rats [3]. In man the latter effect is attributed to a drug-induced hypoglycemia [4]. Species-specific functional and morphological alterations in rat pancreatic B-cells have been reported after sub-chronic administration of cyproheptadine [5, 6]. Unlike alloxan- (mesoxalylurea) [7] or streptozotocin-(N-nitrosoglucosamine) [8] induced diabetes mellitus in rats, where complete degeneration and necrosis of the B-cells occur even after single doses, cyproheptadine produces a selective and localized B-cell lesion. Morphological alterations are restricted to a depletion of secretory granules and excessive dilation of the rough endoplasmic reticulum, which are accompanied by hyperglycemia [6].

In the present study, the effects of cyproheptadine medication on the B-cells of hypophysectomized rats were investigated. Although the possible involvement of pituitary hormones in the genesis of the lesions would still remain entirely hypothetical, the failure to produce lesions in hypophysectomized animals would indicate that the B-cell changes do not simply result from the direct toxic action of cyproheptadine.

Materials and Methods

Twenty male albino rats (OFA Sandoz specific pathogen-free strain) weighing 170–250 g were hypophysectomized [9]. Ten others underwent a sham operation. Following surgery, animals were caged separately and allowed food (NAFAG® from Nafag AG, Gossau, Switzerland) and 5% glucose solution ad libitum. On the fourth post-operative day, a 10-day period of cyproheptadine administration began (see Table 1), following which rats were taken in random order after overnight fasting and exposed briefly to ether. 1 ml blood samples were collected from the retro-orbital vein for determination of glucose by the potassium ferricyanide-ferrocyanide method on a Technicon Autoanalyzer [10]. Rats were then killed by exsanguination while under deep ether narcosis. Small pieces of

Table 1. *Grouping and treatment of rats*

Operative Procedure	Once daily treatment (given by gastric intubation)	Number of Rats
Hypophysectomized (Hy)	5 ml Tragacanth only	10
Hypophysectomized (Hy)	40 mg/kg/day cyproheptadine[a] (CPH) suspended in 5 ml Tragacanth	10
Sham-operated (S.O.)	40 mg/kg/day cyproheptadine (CPH) suspended in 5 ml Tragacanth	5
Sham-operated (S.O.)	5 ml Tragacanth only	5

[a] Periactin® Merck Ltd., Darmstadt, W. Germany

pancreas were taken and prepared for electron microscopy by a method similar to that of Wold *et al.* [5]. The remainder of the gland was fixed in Bouin's solution, embedded in paraffin and stained with hematoxylin and eosin or aldehyde fuchsin. Following removal of the pancreas the sella turcica of each hypophysectomized rat was examined for the presence of pituitary fragments. The pancreas and blood sample of any rat showing remaining fragments were discarded.

To estimate the relative pancreatic islet size, the maximum diameters of all islets on one aldehyde fuchsin-stained section were measured with a reticule in the eyepiece of the light microscope in 4 rats from each of the two hypophysectomized groups and 5 rats from the sham-operated groups. Examinations were made "blind", with slides in random order.

Results

Histology (Hematoxylin and Eosin and Aldehyde Fuchsin)

Pancreatic islets of both untreated groups and the treated hypophysectomized group had normal morphology with a good degree of B-cell granulation (Fig. 1). Islets of the sham-operated rats treated with cyproheptadine had the typical cytoplasmic vacuolization of most B-cells previously reported [5]. These islets also had a severe degree of B-cell degranulation (Fig. 2).

Measurement of Islet Diameter

The results are given in Table 2. Medication of sham-operated rats resulted in a significant increase in mean islet diameter of 30%. No increase was seen in hypophysectomized rats receiving identical treatment.

Ultrastructure

Islet cell ultrastructure of non-treated hypophysectomized or sham-operated rats conformed to the normal morphology previously described by Lacy [11].

In sham-operated rats treated with cyproheptadine, alterations in B-cell morphology, typical of those previously described [5, 6], were observed. There was marked dilation of rough endoplasmic reticulum, with formation of vesicles containing an electron-dense granular material (Fig. 3). These vesicles were, in some cases, studded along their surface with ribosomes. In many B-cells, vesicles showed fusion and vacuoles attaining nuclear size were observed. Only a few secretory granules were seen. The morphology of A-cells appeared normal.

Islet cell ultrastructure of hypophysectomized rats treated with cyproheptadine was similar to that of the untreated groups (Fig. 4). No evidence of changes in the rough endoplasmic reticulum or secretory granule content of B-cells was seen.

Blood Glucose Estimations

Both sham-operated groups became hyperglycemic as a result of the 5% glucose they drank, (Table 3). The hyperglycemia was significantly greater in the group that received cyproheptadine, a finding which is in agreement with that of other investigators [5, 6]. In contrast, both hypophysectomized groups were normoglycemic, even though they drank more 5% glucose than the sham-operated rats. This is probably

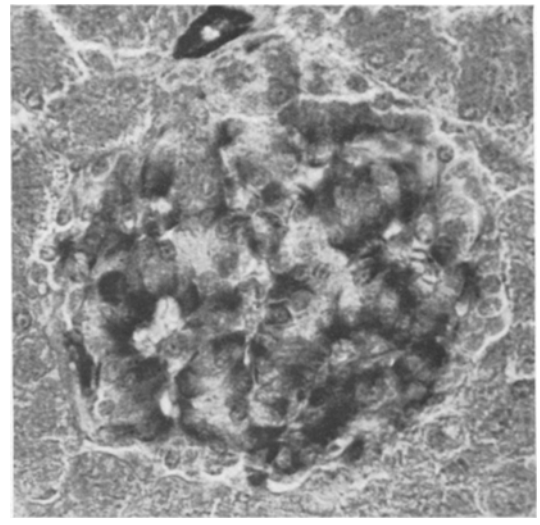

Fig. 1. An islet of Langerhans from a hypophysectomized rat treated with 40 mg/kg/day cyproheptadine orally for 10 days. B-cells show marked granulation, similar to those seen in untreated rats. Aldehyde-fuchsin stain. ×400

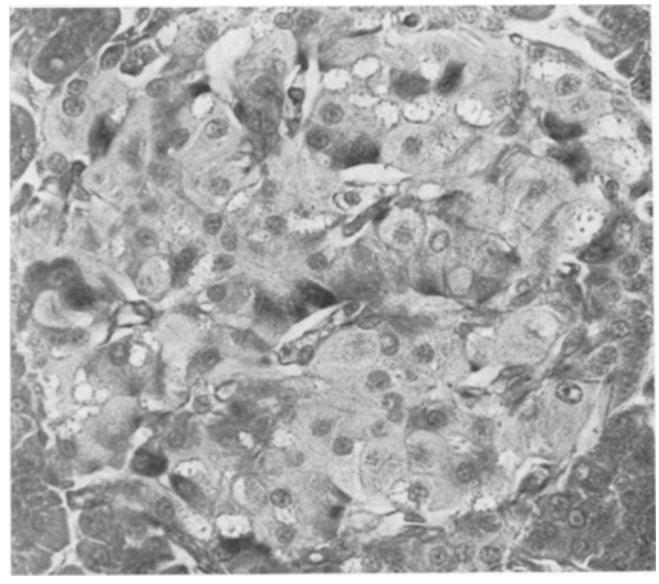

Fig. 2. An islet of Langerhans from a sham-operated rat treated with 40 mg/kg/day cyproheptadine orally for 10 days. Most B-cells show complete degranulation. A trace of positive staining occurs in occasional cells. Intracytoplasmic vacuoles are present in a number of B-cells. Aldehyde fuchsin stain. ×400

explained by the alterations in carbohydrate metabolism that are known to follow hypophysectomy. In addition, there was no drug-induced elevation of blood glucose levels in hypophysectomized rats compared with the hypophysectomized rats receiving no treatment.

Discussion

Pancreatic B-cell lesions, with ultrastructural and light microscopic characteristics typical of those previously reported to occur in rats after cyproheptadine treatment, were reproduced in rats with an intact pituitary. Significant increase in pancreatic islet size and resting blood glucose levels also occurred in these rats. None of these effects could be produced in hypophysectomized rats receiving identical treatment.

Few similarities exist between cyproheptadine-induced pancreatic B-cell lesions and those produced by other classical, chemical diabetogenic agents, such as alloxan or streptozotocin [5, 6]. Cyproheptadine-

Table 2. *Effect of 10 days' oral cyproheptadine (CPH) administration (40 mg/kg/day) on the size of islets of Langerhans of sham-operated (S.O.) and hypophysectomized (Hy) rats*

Group	Number of rats examined	Number of Islets measured	Mean size of islets in units of the reticule (± standard error)
Hy/Control	4	185	1.88 (±0.085)
Hy/CPH	4	95	1.60 (±0.099)
S.O./CPH	5	156	2.42 (±0.118)a
S.O./Control	5	133	1.80 (±0.102)

a = by Student's t-test $P<0.001$ versus values for other 3 groups. All other intergroup differences not significant.

Table 3. *Effect of 10 days' oral cyproheptadine (CPH) administration (40 mg/kg/day) on resting blood glucose levels of sham-operated (S.O.) and hypophysectomized (Hy) rats*

Group	Number of rats	Mean blood glucose in mg/100 ml ± standard error		P value[a]	
Hy/Control	4	118 (±12)	not significant	<0.01	<0.05
Hy/CPH	4	101 (± 9)			
S.O./CPH	5	203 (±17)	(<0.05)		
S.O./Control	5	158 (± 6)			

[a] values examined by Student's t-test.

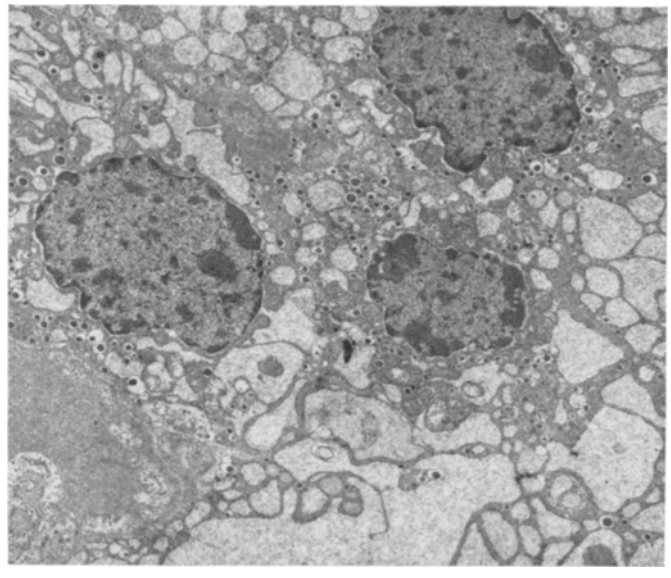

Fig. 3. B-cells from a sham-operated rat treated with 40 mg/kg/day cyproheptadine orally for 10 days. A marked dilation of rough endoplasmic reticulum to form cytoplasmic vesicles and vacuoles is present. These structures appear filled with an electron-dense granular material. Only few secretory granules remain. Lead citrate and uranyl acetate staining. ×7000

induced lesions do, however, resemble those occurring in rat pituitary thyrotropes after thyroidectomy and in gonadotropes following castration [12]. The changes produced in the cells of the adenohypophysis under such conditions are believed to represent morphological adaptations to chronic over-stimulation by their respective hypothalamic releasing hormones. This suggests the possibility that the B-cell lesions produced by cyproheptadine might also arise as a result of chronic over-stimulation by a humoral factor. Certainly growth hormone is capable of stimulating the synthesis and secretion of insulin by B-cells [13, 14, 15] and lesions seen in these cells after its administration are considered to represent an over-stimulation [15]. When given to partially depancreatized dogs, it produces a loss of secretory granules with subsequent vesiculation and vacuolization of the rough endoplasmic reticulum of pancreatic B-cells [17], changes which are similar in several respects to those produced by cyproheptadine in rats.

The recently discovered humoral factor of the rat lateral hypothalamus, which gains access to peripheral blood and is capable of directly stimulating insulin release from pancreatic islets [18], could be considered another hormone which is potentially capable of overstimulating the B-cell. The finding of such a factor in the region of the hypothalamus where the feeding centre is located [19] suggests a close relationship between feeding behaviour and insulin secretion. Cyproheptadine has been shown to increase food intake in man [2], rats [3] and cats [20] and in the latter it was found that this phenomenon was associated with elevated electrical activity in the feeding centre of the lateral hypothalamus [20], the same area in which the islet stimulating factor was found in rats. These facts suggest that the possibility of a drug-induced increase in hypothalamic islet stimulating factor secretion in rats should be considered as a possible mechanism for the production of B-cell lesions occurring after cyproheptadine administration. If this were the case, the failure to produce B-cell effects in hypophysectomized rats treated with cyproheptadine would suggest that the islet stimulating factor normally gains access to the peripheral blood via the pituitary.

As the difference between hypophysectomized and sham-operated rats is much more than a simple absence of pituitary hormones, the involvement of growth hormone or lateral hypothalamic islet stimulating factor in the genesis of the B-cell lesions seen after cyproheptadine medication still remains entirely hypothetical. Nevertheless, this study has stressed the importance of an intact pituitary for the production of cyproheptadine-induced B-cell lesions in rats and demonstrates that the effect of the drug on these cells is not simply a direct one. Further work is in progress to assess to what extent, if any, a possible drug-induced pituitary or hypothalamic stimulation could explain these results.

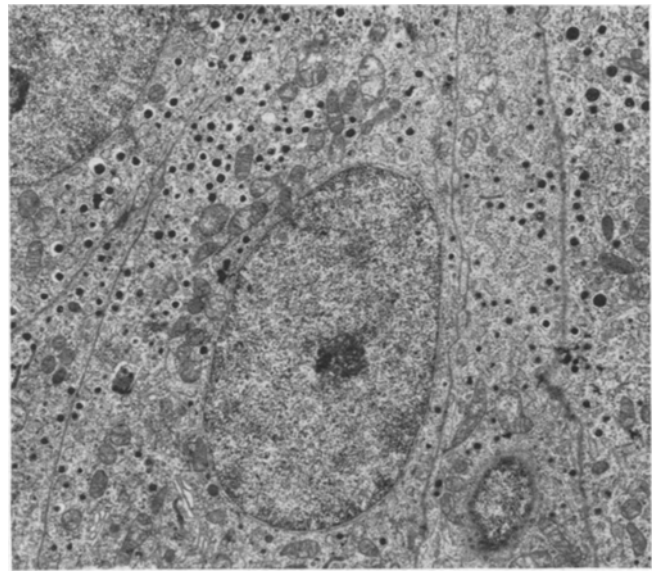

Fig. 4. B-cells from a hypophysectomized rat treated with 40 mg/kg/day cyproheptadine orally for 10 days. Ultrastructural morphology is similar to that of untreated rats. Note the presence of many dark secretory granules and mitochondria. Lead citrate and uranyl acetate staining. $\times 7000$

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