FURTHER STUDIES ON THE RELATIONSHIP OF GLUCAGON TO THE ALPHA CELL OF THE PANCREAS’
SERGIO A. BENCOSME, S. MARIZ, AND J. FREI

Abstract

The relationship of the morphology of the alpha cell and the amount of glucagon present in the pancreas of dogs and rabbits treated with drugs reputed to be toxic for the alpha cell has been further investigated. A summary is given of several experiments dealing with the lack of alpha cell damage in several species following the administration of drugs reputed to be toxic for the alpha cell. It is concluded that glucagon is produced by the alpha cell of the pancreas, and that much of the confusion on this subject probably, results from a failure to recognize the technical difficulties involved in obtaining accurate morphological definition of the structure of the alpha cell at the time the tissue was excised from the body.

Introduction

From recent work we have presented evidence suggesting that glucagon is intimately related to the alpha cells of the pancreas (2, 5, 6, 7, 8) but there are still many differences of opinion on this subject. Because of this we believe it would be timely to report our observations relating glucagon content of the pancreas to the morphology of the alpha cells in a number of animal species subjected to different drugs toxic to the alpha cell.

Methods and Results

A total of 15 dogs and 55 rabbits were used. Healthy mongrel dogs weighing between 20 and 40 lb. were fed raw tripe; New Zealand White rabbits weighing between 2.5 and 5.5 kg. were fed Purina Rabbit Chow; all animals had free access to water.

Alloxan, when used, was injected as a 5% solution intravenously, 75 mg./kg. for dogs and 150 mg. /kg. for rabbits; synthalin A* was injected as a single subcutaneous injection, using a 1% solution. Blood glucose was determined by a micromodification of the Folin-Wu method (21).

Animals were killed with an overdose of nembutal. In dogs, glucagon extracts were obtained separately from the uncinate process and the remainder of the pancreas. As in similar types of experiments, sections were taken from the uncinate process and the tail of the pancreas in order to confirm the absence of alpha cells in the uncinate process of the pancreas (6). In rabbits, a section from the splenic end of the pancreas was used for histological examination while glucagon was extracted from the remainder of the pancreas. Tissues were fixed in Zenker formol. Routine sections cut at 2.5 microns were stained with a modification of Masson’s trichrome (2,3), Gomori’schrome alum hematoxylin (3), and with Gomori’s aldehyde fuchsin stains (20).

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*Bios Laboratories Inc., 17 West 60th Street, New York, N.Y., U.S.A.

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Glucagon was extracted following the procedure of Best et al. (9) and tested according to the method of Staub and Behrens (23). The details of these procedures, as applied in this laboratory, have been given elsewhere (6).

(Group 1) Rabbit Control
The pancreas of four untreated rabbits were used as controls. Individual extracts from 0.5 g. of pancreas were tested once, after which the remainder of these four extracts was pooled and the equivalent of 1 g. of pancreas tested in triplicate.
Detailed description of the cytology of the alpha and delta cells in the rabbit is to be found in earlier reports (1, 2, 3, 4). When tested, the pancreatic extracts of the control rabbits elicited the expected hyperglycemia. This was slightly higher when the equivalent of 1 g. instead of 0.5 g. of pancreas was used (Figs. 1 and 2).

(Group 2) Rabbit-Synthalin A 6 mg.
Twenty-six rabbits received 6 mg. of synthalin A/kg.; 11 died and were discarded; the remaining 15 were killed between 8 and 30 hours after synthalin A injection. Some of the alpha cells of these rabbits showed hydropic changes (Figs. 3 and 4), as described by Davis (14). The degree of alpha cell damage was graded from 0 to 3 plus. The latter grade was used when about 60% of the alpha cells were hydropic. This percentage corresponded to the maximum extent of alpha cell damage found in synthalin-A-treated rabbits. If about 30% of the alpha cells were hydropic, the case was considered a 2 plus, and 1 plus if only a few hydropic cells were present. Six animals had 0, four had 1, two had 2, and three had 3 plus lesions of their alpha cells. As seen in Fig. 1, except for rabbit S8, the hyperglycemic activity of these pancreatic extracts was comparable to that of the controls, irrespective of the degree of alpha cell lesions. Because of the hyperglycemic activity present in the pancreatic extracts with 0, 1, 2, and 3 plus alpha cell lesions, it was considered that bio-assay of all available extracts showing less than a 3 plus lesion would be unlikely to reveal any significant information, and assays on these were not done.

(Group 3) Rabbit-A Iloxan, Synthalin A 6 mg.
Eleven alloxanized rabbits received 6 mg. of synthalin A/kg. on the fourth day after alloxan injection. Twenty-four hours after this injection, the four survivors were killed.

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FIG. 1. Blood sugar changes expressed in mg. % in cats injected with extracts from the pancreas of normal rabbits and of others treated with synthalin A and alloxan plus synthalin A. Dotted lines are used to facilitate the tracing out of curves. In each case extract of 0.5 g. of pancreas was assayed in a cat.

FIG. 2. Blood sugar changes expressed in mg.% in cats injected with extracts from the pancreas of dogs and rabbits treated as indicated in the figure. U-uncinate process; B—body of pancreas. Dotted lines and empty circles are used to facilitate the tracing out of curves. In each case extract of 1 g. of pancreas was assayed in a cat.
FIG. 3 Hydropic degeneration in the alpha cell of a rabbit which received 6 mg. of synthalin A., and which was killed 30 hours after. No changes are present in the beta cells. Gomori's hematoxylin. X 1300

FIG 4. Normal and hydropic alpha cells in the pancreatic islet from an alloxan diabetic rabbit which received 6 mg. of synthalin A, and which was killed 24 hours after. Alpha cells stain black. Delta cells are pale-stained, non-granular and unaffected; beta cell are not present. Masson's trichrome. X 1300

FIG. 5. Pancreatic islet of an alloxan and cobalt-treated dog. composed mostly of alpha and delta cells. Few densely stained alpha cells (a) are seen. Gomori's hematoxylin. X384

FIG. 6. Small area from Fig. 5 showing well-granulated alpha cells and some of the non-granulated, dark-stained alpha cells (a). X950
Islet tissue was almost completely absent from the pancreas of rabbit T40, but when found, it was composed of few (5-10) normal-appearing well-granulated alpha cells. The pancreas of rabbit U11 showed numerous hydropic alpha and normal delta cells (Fig. 4); beta cells were seen only occasionally. Complete serial sections of the biopsies of rabbits T38 and T47 disclosed only fatty tissue. Moderate hyperglycemic activity was present in extracts from rabbits T40 (0 lesion) and U11 (3 plus lesions) but marked hyperglycemic activity was obtained with rabbits T38 and T47.

(Group 4) Rabbit-Synthalin A .10 mg.
Six rabbits having received 10 mg. of synthalin A/kg. according to the method of Fodden and Read (19) were autopsied immediately after death. The rabbits died between 5 and 11 hours after synthalin A injection. Since no alpha cell lesions were noticed, their pancreatic extracts were pooled before testing. The pooled extract showed a hyperglycemic activity comparable to that of the controls (Group 1) (Fig. 2).

(Group 5) Rabbit-A Iloxan, Synthatin A 10 mg.
Five alloxanized rabbits received 10 mg. of synthalin A/kg. on the fourth day after alloxan injections, and were treated thereafter as in Group 4. The rabbits died between 3 and 10 hours after synthalin A injection. Since no alpha cell lesions were noticed, their pancreatic extracts were pooled before testing. The pooled extract showed a hyperglycemic activity comparable to that of the controls (Group 1) (Fig. 2).

(Group 6) Rabbit-Cobalt
Three rabbits were killed 24 hours after having received 40 mg. of cobalt chloride/kg. as a single intravenous injection. Pancreatic extracts from these three rabbits were pooled before testing because no change was found in the alpha cells. When tested, this pooled extract showed a hyperglycemic activity comparable to that of the controls (Group 1) (Fig. 2).

(Group 7) Dogs-Synthalin A
Three dogs, having received 6 mg. of synthalin A/kg., were killed 24 hours later. Three other dogs, having received 20 mg. of synthalin A/kg., were autopsied immediately after death, which occurred respectively at 9, 10, and 11 hours after injection. Another three dogs, having received 25 mg.of synthalin A/kg., were all found dead 18 hours later. Glucagon extractions were not done from the last three dogs, but the pancreas was studied morphologically.
The alpha cells of none of these dogs were altered, neither was the content of glucagon of the pancreatic extracts made (Fig. 2).

(Group 8) Dogs- Alloxan, Cobalt
Six dogs received, following the method described by Carter (11), two doses of 75 mg./kg. of alloxan 24 hours apart. They were then injected hourly for 3 hours with 300 mg. of Cobalt chloride dissolved in 25 cc. of distilled water. Because the histology of the pancreas of these six dogs was similar, the extracts from the uncinate process and from the body of the pancreas were pooled from the respective areas and the pooled extracts tested separately. No alterations were found in the alpha or delta cells nor in the glucagon content of the pancreas of these animals (Table I). Not infrequently, dark non-granular cells with the
staining characteristics of an alpha cell were seen in the islet of alloxan- and cobalt-treated dogs. These cells, which may be mistaken for degenerating alpha cells following cobalt treatment were equally seen following alloxan injections, and in lesser numbers in untreated dogs (Figs. 5 and 6).

| TABLE I |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **GROUP 8** BLOOD SUGAR RESPONSE OF FASTING ANAESTHETIZED NORMAL CATS TO INTRAVENOUS INJECTION OF PANCREATIC EXTRACTS FROM SIX ALLOXAN- AND COBALT-TREATED DOGS. (A) FROM THE BODY OF THE PANCREAS CONTAINING THE ALPHA CELLS. (B) FROM THE UNTANATE PROCESS WHICH IS DEVOID OF ALPHA CELLS  |
| **Material injected** | **Dose, g.** | **Before injection** | **5** | **10** | **15** | **20** | **25** |
| A. Pooled extract of the alpha cell-containing portion of pancreas from six dogs | 1 | 108 | 188 | 178 | 164 | 135 | 123 |
| 1 | 94 | 149 | 181 | 153 | 137 | 118 |
| B. Pooled extract of the pancreatic tissue devoid of alpha cells (untanate process) from the same six dogs | 1 | 114 | 114 | 111 | 111 | 106 | 100 |
| 1 | 100 | 100 | 97 | 100 | 118 | 112 |

(Group 9)

In other experiments, which will not be presented in detail, an attempt was made to confirm, by repeating their experiments as closely as possible, the work of others who claimed to have produced alpha cell damage; in this group glucagon was not assayed. A total of 18 dogs, 46 rabbits, 48 rats, and 38 guinea pigs were used. A summary of our observations follows: cobalt chloride, synthalin A, and thiodiazol-5-imid-4-aminobenzolsulphon-2-isopro-pyl-1-3,4(I.P.T.D.) were given to rats; I.P.T.D., Orinase, and cobalt were given to dogs; I.P.T.D., Orinase, nickel chloride, and sodium-diethyl-dithiocarbamate were given to rabbits; Orinase and synthalin A were given to guinea pigs. In all these experiments on different animals only guinea pigs treated with synthalin A showed some hydropic changes in their alpha cells. This lesion was, however, very inconstant, and of much lesser intensity than when it is produced by administering cobalt.
Discussion

Hydropic degeneration of the alpha cells occurred after the administration of synthalin A in the rabbit, but not in the dog. Cobalt chloride produced no changes in the alpha cell of dogs or rabbits. This is in agreement with previous work where we showed that severe damage could be induced with cobalt in the alpha cell of guinea pigs, but not in that of rabbits (2). A more general agreement on the structural alterations, or lack of them, in the alpha cell under the influence of various drugs is necessary if progress is to be made in our knowledge of its physiological function. Very recently, a relationship of the alpha cell function with atherosclerosis has been postulated. It has been stated that alpha cells produce pancreatic elastase (11) and a hormone which regulates the cholesterol metabolism (10). Both these hypotheses were made on the basis of lesions occurring in the alpha cells of cobalt-treated dogs and rabbits, whereas, as pointed out above, no demonstrable morphological damage occurs in the alpha cells of dogs, rabbits, and rats with cobalt.

As anticipated, no reduction in the glucagon content of the pancreas of our experimental animals was observed since only minimal damage, or no damage, occurred in the alpha cells. These results are in agreement with those reported earlier where we showed that the hyperglycemic activity of pancreatic extracts was absent when the extracts were completely devoid of alpha cells (6, 7, 8) or if at least 90% of these cells were severely damaged (5). They are at variance, however, with those of Fodden and Read (19), who found no glucagon in the pancreas of synthalin-A-treated rabbits, whereas this substance persisted in the pancreas of cobalt-treated ones despite the fact that alpha cell lesions were present in both groups. The hyperglycemic activity found by these authors in the pancreas of cobalt-treated animals is better explained in the light of Fodden’s more recent paper (15), in it he concluded that no lesions occur in the alpha cells of cobalt-treated rabbits. No detailed discussions were given by Fociden and Read (19) of the intensity and incidence of the alpha cell lesion of their synthalin-A-treated rabbits. It might be suggested that the difference between the results obtained by these authors and by ourselves was due to the fact that we used cats instead of rabbits for testing the extracts. In our hands, rabbits used as test animals showed false negative results when cross-checked with cats.

It seems that the lack of uniformity in the results of applying special methods of histopathological technique by different investigators, together with inro- complete reporting of observations and brevity of discussion of the morphoere logical changes of the alpha cell, has been responsible in several instances for the controversy over whether the alpha cell is the site of origin of glucagon (5, 12, 15, 19, 22, 25).

So far we have not been able to provide conclusive evidence that glucagon is produced by the alpha cell. However, the available literature, as well as the work from this laboratory are best interpreted if one considers the alpha cell of the pancreas as the site of origin of pancreatic glucagon.
References

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