Upsala Journal of Medical Sciences

ISSN: 0300-9734 (Print) 2000-1967 (Online) Journal homepage: informahealthcare.com/journals/iups20

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L. Grimelius, H. Johansson & A. Segerström

**To cite this article:** L. Grimelius, H. Johansson & A. Segerström (1972) Pancreatic Islet Cells in Experimental Hypo- and Hyperparathyroidism in Rats, Upsala Journal of Medical Sciences, 77:3, 140-142, DOI: 10.1517/03009734000000021

**To link to this article:** https://doi.org/10.1517/03009734000000021

Published online: 02 Mar 2011.

Upsala J Med Sci 77: 140–142, 1972

# Pancreatic Islet Cells in Experimental Hypo- and Hyperparathyroidism in Rats

L. GRIMELIUS, H. JOHANSSON and A. SEGERSTRÖM

*From the Institute of Pathology and the Department of Surgery, University of Uppsala, Sweden*

## ABSTRACT

**The hypocalcaemic effect of glucagon is well documented in the literature. In this investigation the pancreatic islet cells were studied in hypocalcaemic (parathyroidectomized) and hypercalcaemic (parathyroid hormone treated) rats. Observations were made after 1 and 8 weeks of hypocalcaemia and after 3 days of hypercalcaemia. No qualitative or quantitative changes in the islet cells were observed. The results are discussed.**

## INTRODUCTION

The hypocalcaemic effect of glucagon is well documented in the literature and it has been shown in animal experiments that administration of glucagon induces hyperplasia of the parathyroid glands (11). The question whether the endocrine function of the pancreas can be affected by altering the function of the parathyroids, on the other hand, seems to have received little attention in the literature (see (11, 12)).

The aim of the present study was to ascertain whether the pancreatic islet cells, especially the glucagon-producing $\alpha_2$ cells, show any qualitative or quantitative changes under hypo- and hypercalcaemic conditions.

## MATERIAL AND METHODS

Thirty-six adult male rats of the Sprague-Dawley strain, with an initial weight of 200 to 250 g, were used.

Sixteen rats were parathyroidectomized by fine needle diathermy (8, 9). Seven of these rats were killed after 1 week and 9 after 8 weeks.

Four rats were treated with parathyroid hormone (Para-Thor-Mone®, 100 USP units per ml, Ely Lilly) for 3 days. The hormone was injected bidiurnally by the subcutaneous route and the dose was 800 USP units per 100 g body weight and day.

In all groups control animals were used for comparison (see Tables I–III). All animals were fasted for 18 hours before they were killed but received water *ad libitum*. They were killed at the same time of day with an overdose of ether.

The parathyroid function was evaluated by means of repeated determinations of the serum calcium level by flame photometry (Eppendorf). Preoperatively the mean serum calcium value was 5.0 mEq/l (S.D. 0.57). Only rats with a serum calcium decrease postoperatively to 4.1 mEq/l or less were used (9). The serum calcium level in the control animals remained unchanged during the experimental period. The initial body weight and weight increase of the parathyroidectomized and parathyroid hormone treated rats did not differ from those of the control animals.

*Fixation*

Pancreatic tissue was fixed in 10% formalin for 24 hours or in Bouin fluid for 22 hours.

*Staining procedure*

Adjacent 5 $\mu$ thick deparaffinized sections were stained with (*a*) van Gieson stain; (*b*) Davenport alcoholic silver nitrate stain as modified by Hellerström & Hellman (6); (*c*) variant I of the Grimelius silver nitrate stain (5). An 0.01% solution of the silver nitrate stain was used instead of 0.03%; the reason for this reduction was to prevent grey or grey-green granulation of the centrally located $\beta$ cells, as sometimes appears in the pancreatic islets of rats; (*d*) Gomori aldehyde fuchsin stain mainly as modified by Maske (10) with ponceau fuchsin as a counterstain (7).

*Differential counts* were carried out on formalin-fixed sections stained with the two silver stains and the Gomori stain, in a binocular light microscope with a magnification of $\times$ 1 250 (immersion lens) and with the aid of a movable stage and a squared grid placed in one of the eyepieces.

The sections were systematically examined and the islets differentially counted in the order in which they occurred. In the adjacent sections the study began in the same area. Only cells with nuclei or nuclear fragments were counted. The $\alpha_1$ cells were counted in the Davenport stain, the $\alpha_2$ cells in the Grimelius stain and the $\beta$ cells in the Gomori stain. At least 2 000 islet cells were counted in each case and stain.

## RESULTS

Light microscopy revealed no structural or tinctorial changes in the pancreatic islets in either

Tables I and II. *The percentual distribution of the different cell types in pancreatic islets of parathyroidectomized rats (PTE) in comparison with controls. Table I refers to animals killed 1 week and Table II 8 weeks postoperatively*

$\alpha_1$ cells were counted in the Davenport silver stain, $\alpha_2$ cells in the Grimelius silver technique and $\beta$ cells in the Gomori stain. At least 2 000 cells were counted in each case and stain

| | $\alpha_1$ cells in % | | $\alpha_2$ cells in % | | $\beta$ cells in % | |
|---|---|---|---|---|---|---|
| | PTE | Controls | PTE | Controls | PTE | Controls |
| Table I. (*1 week*) | | | | | | |
| | 4.7 | 7.6 | 38.1 | 30.7 | 59.2 | 61.6 |
| | 6.6 | 6.7 | 36.6 | 28.8 | 54.6 | 62.3 |
| | 6.5 | 6.4 | 40.3 | 35.3 | 55.0 | 57.0 |
| | 5.1 | 6.2 | 40.0 | 33.9 | 56.5 | 60.6 |
| | 5.9 | 6.9 | 36.9 | 39.4 | 59.6 | 52.1 |
| | 6.5 | 6.3 | 29.3 | 36.7 | 62.2 | 60.5 |
| | 8.0 | 4.1 | 37.7 | 37.2 | 58.0 | 60.6 |
| $M^a$ | 6.19 | 6.31 | 36.99 | 34.57 | 57.87 | 59.24 |
| S.E.M.[b] | 0.41 | 0.41 | 1.39 | 1.41 | 1.03 | 1.35 |
| Diff.[c] | −0.13 | | +2.42 | | −1.37 | |
| *t*-value | −0.22 | | +1.22 | | −0.81 | |
| Level of significance | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |
| Table II. (*8 weeks*) | | | | | | |
| | 7.2 | 5.1 | 30.3 | 26.0 | 63.1 | 70.6 |
| | 3.2 | 6.1 | 24.9 | 33.4 | 71.8 | 63.4 |
| | 4.7 | 4.4 | 24.9 | 26.6 | 69.3 | 72.9 |
| | 7.2 | 7.1 | 25.4 | 34.8 | 64.5 | 64.6 |
| | 3.4 | 8.5 | 35.9 | 28.8 | 62.3 | 65.2 |
| | 7.0 | | 39.2 | | 53.1 | |
| | 6.0 | | 33.4 | | 63.3 | |
| | 6.2 | | 30.4 | | 66.6 | |
| | 5.9 | | 26.7 | | 68.7 | |
| $M^a$ | 5.64 | 6.24 | 30.12 | 39.92 | 64.74 | 67.34 |
| S.E.M.[b] | 0.51 | 0.73 | 1.73 | 1.78 | 1.81 | 1.86 |
| Diff.[c] | −0.60 | | +0.20 | | −2.60 | |
| *t*-value | −0.67 | | +0.08 | | −1.00 | |
| Level of significance | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |

[a] Mean value.
[b] Standard error of the mean value.
[c] Difference between $M_{PTE}$ and $M$ Control.

the hypo- or hypercalcaemic rats. Neither were any changes observed in the exocrine pancreatic tissue.

The results of the differential counts showed that the percentual distribution of the three types of cell in the pancreatic islets of the experimental animals did not differ significantly from that in the control animals (see Tables I–III).

## DISCUSSION

Paloyan et al. (12) showed that 8 weeks of glucagon administration to rabbits resulted in hyperplasia of the parathyroid glands in 9 of 11 animals. Marked α cell degranulation was also observed in the pancreatic islets in these animals treated with glucagon. Further evidence of a relationship between the pancreatic islets and the parathyroid glands was presented by Paloyan et al. (12) in their retrospective review of a human autopsy material, where hypertrophy and hyperplasia of the pancreatic islets were found in 12 of 15 cases with parathyroid tumour. Our studies in the rat provide no proof of a relationship between these endocrine organs, but neither do they exclude this possibility.

A species difference may explain the absence of an effect of experimental hypo- and hyperparathyroidism on the pancreatic islet cells, but other explanations are possible. The hypercalcaemic period in our rats may have been too short to affect the pancreatic islet cells qualitatively or quantitatively. The duration of the hypocalcaemia in the rats of one of our experimental series was 8 weeks. The absence of any effect on the pancreatic islets may be due to the hypocalcaemic state, since Stern & Bell (13) consider that glu-

Table III. *Percentual distribution of the different cell types in pancreatic islets of rats treated with parathyroid hormone (PH) for 3 days*

The control animals were given saline solution instead of hormone. For further details see text to Tables I and II

| | $\alpha_1$ cells in % | | $\alpha_2$ cells in % | | $\beta$ cells in % | |
|---|---|---|---|---|---|---|
| | PH | Controls | PH | Controls | PH | Controls |
| | 6.1 | 6.2 | 33.2 | 30.3 | 63.8 | 66.4 |
| | 5.1 | 4.9 | 32.0 | 33.1 | 64.8 | 66.7 |
| | 6.8 | 7.8 | 32.9 | 32.3 | 64.6 | 64.7 |
| | 7.8 | 4.8 | 36.6 | 40.1 | 62.3 | 56.4 |
| $M^a$ | 6.45 | 5.92 | 33.68 | 33.95 | 63.88 | 63.55 |
| S.E.M.[b] | 0.57 | 0.70 | 1.01 | 2.13 | 0.57 | 2.42 |
| Diff.[c] | +0.53 | | −0.28 | | +0.33 | |
| *t*-value | +0.58 | | −0.12 | | +0.13 | |
| Level of significance | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |

[a] Mean value.
[b] Standard error of the mean value.
[c] Difference between $M_{PH}$ and $M$ Control.

cagon is less effective in hypocalcaemia and more effective in the presence of hypercalcaemia. The fact that glucagon influences the serum calcium concentration and the parathyroid glands, regardless of whether the effect is exerted via calciotonin (1, 2, 3, 4) or inhibition of bone resorption (13) or via new formation of bone (14), does not necessarily mean that the opposite is valid. Glucagon has many other effects apart from that on the calcium metabolism. It is possible that other actions, for example those on the carbohydrate, protein and fat metabolism and the intestinal motor function, may make such demands on the $\alpha_2$ cells that changes in the parathyroid function and the serum calcium concentration will affect the glucagon-producing cells of the pancreatic islets to such a small extent that with the method used the changes would not have been detected.

The relationship between hyperparathyroidism and pancreatitis is well documented in the literature. In our studies no signs of inflammation of the pancreas were observed. As mentioned above, the duration of the hypercalcaemia was short.

*Differential counts*

Three differential counts were performed on sections from each experimental and control animal. The $\alpha_1$ and $\alpha_2$ cells were differentially counted in silver stains and the $\beta$ cells in a "granule" stain. The frequency of the cell types was expressed in per cent of counted endocrine cells. In many cases the sum of the three cell types exceeded 100%. As pointed out previously (5), the argyrophil cells are often over-represented in the silver stains and the $\beta$ cells in the "granule" stain used. As in man, a small number of $\alpha$ cells showing a positive silver reaction in both of the silver stains used is found in the rat (Grimelius, unpublished observation). In a small number of cases the sum mentioned above was less than 100%. One reason for this is the presence of degranulated islet cells, which on differential counting will not be included in any of the three groups of cell types. Also contributory to the deviation from the sum of 100 is the error of the method, which depends upon the examiner's experience of differential counting.

Since in this investigation the results of the differential counts in the experimental animals were compared with those in the controls, the over-representation mentioned above as well as of the error the method in differential counting is of less importance.

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*Received April 20, 1972*

Address for reprints:

L. Grimelius, M.D.
Institute of Pathology
University of Uppsala
Box 553
S-751 22 Uppsala 1
Sweden