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Effect of cadmium on the expression of insulin and glucagon in non-diabetic and diabetic male Wistar rats

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Abstract

The increasing incidence of diabetes may involve other causes, environmental pollutants. Among the pollutants potentially involved, several studies show a correlation between exposure to cadmium and the severity and incidence of diabetes, but this association remains controversial. Thus, in order to explore the effects of cadmium on pancreatic functions, 20 male Wistar pubescent non-diabetic and diabetic rats received cadmium sulfate orally for 30 consecutive days. After the exposure period, the rats were euthanized. The pancreas were isolated with a view to making histological sections. In non-diabetic rats, cadmium caused an increase in the secretion of glucagon and a decrease in the release of insulin. In contrast, in diabetic rats, cadmium induced an increase in the secretion of insulin and glucagon.

This study showed that cadmium increased glucagon secretion and caused necrosis and partial degeneration of **ß** cells in non-diabetic rats. On the other hand, in diabetic rats, cadmium induced an increase in insulin and glucagon secretion.

Keywords: Cadmium; Diabetes; Insulin; Glucagon; β-cells; α-cells

1. Introduction

Cadmium is a pollutant introduced into the environment due to the rapid development of modern industries and technologies [1]. High concentrations of Cd are present in crustaceans, bivalve molluscs, oysters, cephalopods and crabs; it is also found in organ meats, such as liver and kidney, in oil seeds, cocoa beans and some wild mushrooms [2]. Foods derived from plants, depending on the level of soil contamination, generally contain higher concentrations of cadmium than meat, eggs, milk and dairy products. Of these, rice and wheat, green leafy vegetables, potatoes, carrots, and celery may contain higher concentrations of the metal than other plant foods. Vegetarians and shellfish consumers may be exposed to a higher cadmium intake than omnivores [3]. One of the main routes of exposure to cadmium in humans is through the consumption of rice [4]. When cadmium enters the body, it is transported into the bloodstream via erythrocytes and albumin, and then accumulates in the kidneys [5], liver and intestine [6] but also in the pancreas [7,8]. The pancreas is made up of two distinct parts: the exocrine pancreas and the endocrine pancreas. The latter constitutes about 2% of the mature pancreas [9]. The endocrine pancreas is made up of the islets of Langerhans. These spherical cell aggregates are mainly made up of four endocrine cell types: α cells, β cells, δ cells and PP cells [10]. α cells represent 15 to 20% of the cells of an islet and secrete the glucagon, which is a hyperglycemic hormone. ß cells represent about 60% of the total pancreatic mass and are responsible for the secretion of the hypoglycaemic hormone insulin.

A dysfunction of the pancreas can cause many diseases, the most common of which is diabetes. Diabetes is a metabolic disorder that occurs when the body is unable to make enough insulin or to use insulin effectively.

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The increasing incidence of diabetes and pre-diabetic conditions could involve, among other causes, environmental pollutants. Epidemiological studies suggest a positive association between exposure to cadmium (Cd) and incidence and severity of diabetes [6,8], but this association remains controversial, some experimental data have shown that cadmium can also improve the ratio of glucose-stimulated insulin release [11]. The aim of this study is to demonstrate the effect of cadmium on the secretion of glucagon and insulin in non-diabetic and diabetic male Wistar rats

2. Material and methods

2.1. Animals and treatment

Twenty young male Wistar rats weighing between 209-279 g were purchased from *the Ecole Normale Supérieure d'Abidjan* (*ENS*). These animals were housed at the Institut Pasteur animal facility in plastic cages and a day / night cycle was maintained (approximately 12 hours of light and 12 hours of darkness) in a ventilated animal facility. Rats were acclimatized for 14 days to their new environment prior to treatment and had free access to sterile distilled water and sterilized standard food. All animals were handled according to guidelines and protocols approved by the Animal Care and Use Committee of Cote d'Ivoire.

Diabetes mellitus was induced in rats using the method described by Baydas *et al.* [12]. After fasting for one day, a single dose of 60 mg/kg body weight of streptozotocin (STZ) (Cayman, USA) diluted in bufferfreshly prepared citrate (0.1 mol was injected intraperitoneally.

Blood glucose was measured as described by Diasy et al. [13] from the tail vein using an Accu Chek Active® glucometer (Roche, Germany). According to Faiyaz and Asma[14], three days after STZ injection, rats with blood glucose levels above 250 mg/dl were considered diabetic and used for experimental studies.

The rats were divided into four groups (control, STZ treated, Cd treated and Cd + STZ treated), each group consisting of five rats. Control and STZ treated groups received distilled water and Cd and Cd + STZ treated groups had distilled water enriched in cadmium sulfate (CdSO4) at 200 mg/L [15,16].

The experiment was carried out for 30 consecutive days. After 30 days of treatment, the rats were euthanized, the pancreas were quickly removed for histological sections.

2.2. Immunohistochemistry staining of insulin

Tissue sections were deparaffinized using xylene (3 x 5 min) and rehydrated successively with 100% and 95% ethanol (2x2min). They were rinsed in distilled water then immersed in a microwave solution and heated 3 successive times for 15 minutes at 80 W in a microwave oven for the unmasking of antigenic sites. The sections were rinsed in PBS - Triton - 100X (0.025%) for 2x2 min and then blocked with 3 drops of RTU normal goat serum (GeneCopoeia, USA) for 30 min. The tissue sections were incubated with primary mouse monoclonal Anti-Insulin antibody (Boster Biological Technology Co, USA) diluted at 1:100 in the normal goat serum for 2 hour at room temperature.

The sections were rinsed in 1 x PBS and the endogenous peroxidase activity in tissues was blocked by incubation in 3% H_2O_2 for 10 min at room temperature. The slides were rinsed in 1 x PBS followed by incubation with 2 drops of RTU biotinylated anti-mouse secondary antibody (Burlingame CA, USA) for 30 minutes at room temperature. The tissues were rinsed in 1 x PBS for 3 x 2 min. The detection was done by incubating the sections with 2 drops of RTU streptavidin-HRP (GeneCopoeia, USA) for 30 minutes at room temperature. The sections were rinsed in 1 x PBS (2 x3 min) and then incubated a room temperature with 2 drops of DAB (GeneCopoeia, USA) working solution during 6 minutes. The signal development was monitor under microscope. The slides were rinsed in distilled water (2 x 2 min) and counterstained by incubating the sections with 2 drops of RTU hematoxylin solution (GeneCopoeia, USA) for 2 minutes. Sections were rinsed in distilled water and mounted using fluoromount-G mounting medium (Southern Biotech, USA).

2.3. Immunohistochemistry staining of glucagon

Tissue sections were deparaffinized using xylene (3 x 5min) and rehydrated successively with 100% and 95% ethanol (2x2min). They were rinsed in distilled water then immersed in a microwave solution and heated 3 successive times for 15 minutes at 80 W in a microwave oven for the unmasking of antigenic sites. The sections were rinsed in PBS - Triton - 100X (0.025%) for 2x2 min and then blocked with 2-3 drops of RTU normal goat serum (GeneCopoeia, USA) for 30 min. The tissue sections were incubated with primary rabbit monoclonal Anti-Glucagon antibody diluted at 1:10 in the normal goat serum for 2 hour at room temperature.

The sections were rinsed in 1 x PBS and the endogenous peroxidase activity in tissues was blocked by incubation in 3% H_2O_2 for 10 min at room temperature. The slides were rinsed in 1 x PBS followed by incubation with 2 drops of RTU biotinylated anti-rabbit secondary antibody (Burlingame CA, USA) for 30 minutes at room temperature. The tissues were rinsed in 1 x PBS for 3 x 2 min. The detection was done by incubating the sections with 2 drops of RTU streptavidin-HRP (GeneCopoeia, USA) for 30 minutes at room temperature. The sections were rinsed in 1 x PBS (2 x3 min) and then incubated a room temperature with 2 drops of DAB working solution (GeneCopoeia, USA) for 6 min DAB solution. is made by mixture of 20 µl of DAB stock solution and 20 µl of stable H_2O_2 solution with 1ml of DAB enhancer buffer). The signal development was monitor under microscope. The slides were rinsed in distilled water (2 x 2 min) and counterstained by incubating the sections with 2 drops of RTU hematoxylin solution (GeneCopoeia, USA) for 2 minutes. Sections were rinsed in distilled water and mounted using fluoromount-G mounting medium (Southern Biotech, USA).

2.4. Microscope observation

The sections were observed under an optical microscope equipped with a device (Humascope Advanced LED, Germany) in the parasitology laboratory of the Institut Pasteur in Côte d'Ivoire

3. Results

3.1. Expression of insulin and glucagon

- No morphological or immunohistochemical difference was found in the β cells of the pancreatic islets of the rats in the control group. Numerous insulin positive labeled β cells were observed on the islets (Figure 1A). The rats in the control group exhibited normal islet-of-Langerhans morphology and architecture. The α cells were found on the outskirts of the islets of Langerhans. Glucagon positive labeled α cells were weak (Figure 1E).
- In non-diabetic rats treated with cadmium, slight degeneration, necrosis of a few β cells and slight degranulation were observed in approximately 30% of the β cells of the pancreatic islets. The number of β cells labeled positive for insulin was slightly lower than that of the control group (Figure 1B). In non-diabetic rats treated with cadmium, the morphology and architecture of the α cells of the islets of Langerhans is not altered, but the number of α cells marked positive for glucagon is greater than that of the control group (Figure 1F).
- In diabetic rats, degeneration, severe degranulation and necrosis were observed in approximately 75% of β cells of pancreatic islets. The number of β cells marked positive for insulin was almost zero in some islands or much lower than that of the control group (Figure 1C and D). On the other hand, the number of insulin-positive β cells in the rats of the diabetic group contaminated with cadmium was higher than that of the rats of the diabetic control group and lower than the control group (Figure 1C). In diabetic rats (diabetic control group and diabetic group contaminated with cadmium), a modification of the architecture of the α cells of the islets of Langerhans was observed. This change is due to an increase in the number of α cells and a localization of α cells in the center of the islet. The number of α cells labeled positive for glucagon is much higher than that of the control group (Figure 1G). On the other hand, the number of α cells marked positive for glucagon in the diabetic group contaminated with cadmium is higher than that of the diabetic control group and considerably higher than the control group (FIgure 1H).

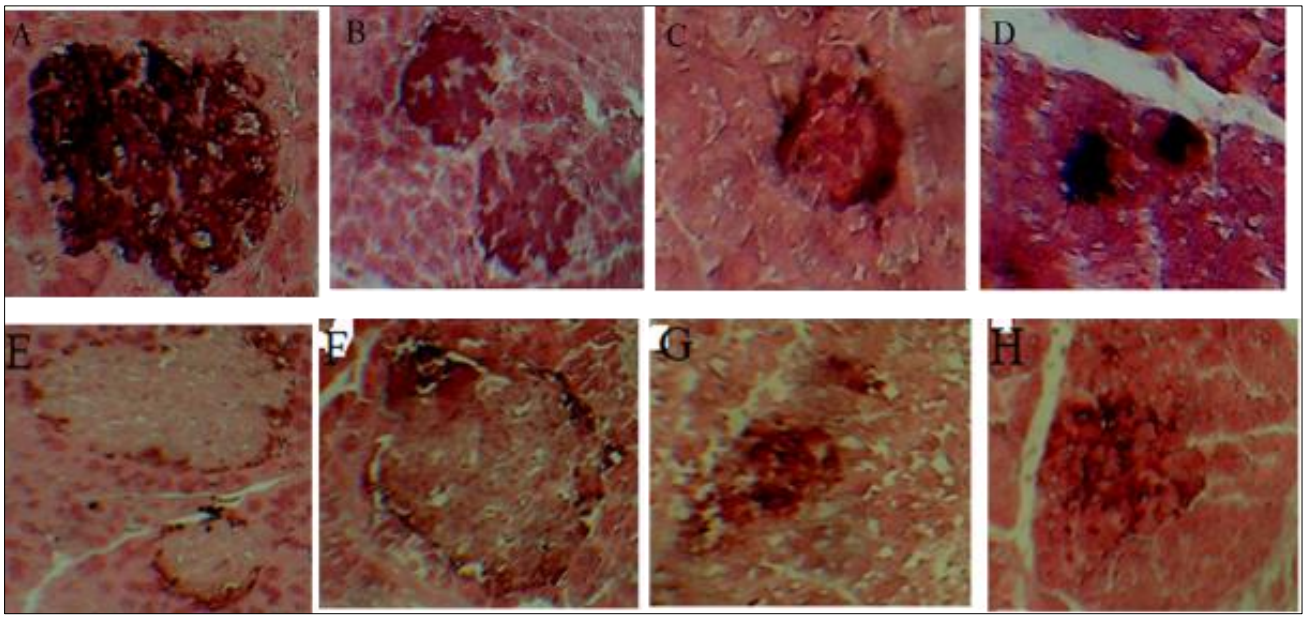

Figure 1 Photographs of insulin-labeled sections of pancreas

A, B, C, D: Photographs of pancreas sections labelled with insulin and glucagon
E, F, G, H: Photographs of pancreas sections marked with glucagon (magnification x 400).
A, E: Ctrl group;
B, F: Cd group;
C, G: STZ group and
D, H: STZ + Cd group.

The comparative study of the secretion of insulin and glucagon in β and α cells in the islets of Langerhans of different groups of rats is summarized in the table below.

Table 1 Semi-quantitative distribution of insulin and glucagon in β and α cells of the islets of Langerhans

Groups	Insulin in β cells	Glucagon in α cells
Ctrl	4	2
Cd	3	3
STZ	1	4
STZ + Cd	1	5

- **(0) absence of insulin in β cells or glucagon in α cells.**

- (1) trace of insulin in β cells or glucagon in α cells.
- (2) low amount of insulin in β cells or glucagon in α cells.
- (3) average amount of insulin in β cells or glucagon in α cells.
- (4) high amount of insulin in β cells or glucagon in α cells.
- (5) very high amount of glucagon in α cells.

4. Discussion

Histological examination of the pancreas revealed damage to islet beta cells and reduced insulin secretion in non-diabetic rats contaminated with cadmium. Indeed, the exposure of β cells to Cd concentrations between 0.1 and 1.0 µmol/L results in cadmium absorption as a function of dose and time [17]. This uptake leads to induction of metallthionein expression, possibly increasing cellular accumulation of cadmium.

Accumulation of cadmium leads to functional impairment of β cell function and inhibition of glucose-stimulated insulin secretion in β cells [17].

There is evidence that SH groups play an important role in the mechanisms leading to the release of stored insulin from pancreatic β cells and that cadmium is also a potent inhibitor of SH [18,19].

In addition, the present study reveals an increase in insulin secretion in diabetic rats contaminated with cadmium. Some researchers have shown in vitro that a concentration of 5μM of Cd in β-pancreatic cells improves the glucose-stimulated insulin release ratio [20]. Other researchers have shown that the accumulation of cadmium in the pancreas caused a 16% increase in beta cell mass in infected adult rats at a dose of 500 μg/ g /day. This increase in beta cell mass could compensate for a lack of insulin secretion by the islets [7].

Concerning glucagon, the results of the present study showed that cadmium stimulates the release of glucagon by α cells in diabetic and non-diabetic rats contaminated with cadmium. In addition, an increase in α cells and a modification of the architecture of the islands of Langerhans were observed in diabetic rats compared to control rats. Numerous studies have also shown a modification of the entire architecture of the islet under these same conditions with the modulation of the two other main cell types of the islet, the α and δ cells, as well as modifications of cell-cell interactions. [21,22].

The hyperglucagonemia of diabetes in humans results from excessive secretion of the hormone and contributes to stimulation of glycogenolysis and gluconeogenesis in the liver, to the establishment and maintenance of hyperglycemia. In type 1 diabetics, the presence of elevated glucagon levels is the result of untreated or poorly monitored diabetes, and is greatest in diabetic ketoacidosis or hyperosmolar coma [23]. In patients with type 2 diabetes, this double endocrine pancreatic dysfunction: a reduction in insulin secretion and less inhibition of glucagon secretion in response to a glucose load, seems to be a very early phenomenon in the sequence of events that lead to the decrease in glucose tolerance [24]. This hyperglucagonemia is due either to insulin resistance of the α cells of the islets of Langerhans, similar to that observed in other cells (muscle cell, hepatocyte, adipocyte) [25], or to desensitization of the α cell by chronic hyperglycemia [26]

5. Conclusion

This study showed that cadmium increased glucagon secretion and caused necrosis and partial degeneration of ß cells in non-diabetic rats. On the other hand, in diabetic rats, cadmium induced an increase in insulin and glucagon secretion.

Compliance with ethical standards

Acknowledgments

We would like to thank the staff of the Anatomy and Pathology Laboratory of the Treichville University Hospital (Abidjan, Cote d'Ivoire).

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The tests carried out on wistar rats in this study were conducted in accordance with the protocol in force at the Institut Pasteur de Côte d'Ivoire.

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