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Studies Concerning the Effect of Glybenclamide, Streptozotocine and Insulin upon Pancreatic Juice Flow and Pancreatic Amylase and Lipase Activity, in Hens

KEYWORDS: pancreatic juice, streptozotocin, glybenclamid, hens

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ABSTRACT *The paper reports the way how the effect of streptozotocin, insulin and glybenclamid administration, reflects on pancreatic juice flow and pancreatic amylase and lipase activity, in hens. The values gathered as result of streptozotocin, insulin and glybenclamid administration revealed the existence in hens of a functional influence between pancreatic hormones and exocrine activity of pancreas. Thus, streptozotocin and glybenclamid have increased the pancreatic juice flow, while insulin reduces flow secretion of pancreatic juice. In all cases was registered an increasing of the TP (total protein), but without obvious signification. Regarding the effect of these substances it's been established that insulin has determined the most intensive amylase activity of pancreatic juice, while glybenclamid reduces the activity of pancreatic amylase. The activity of pancreatic lipase was reduced by insulin, as well as by glybenclamid. Streptozotocin was the only who increased the pancreatic amylase and lipase activity.*

INTRODUCTION

In hens, the endocrine pancreas presents a different structure of α and β cell populations compared to mammals pancreas, these morphological particularities having functional consequences reflected by a raised level of glycemia in hens, compared with the values of this parameter in mammals (Dojana et al, 2000). Also, the exocrine pancreas is very good represented in hens, the pancreatic juice having, at this species, the same enzymatic equipment as in mammals, but with a higher secretion rate. The pancreas, trough hid endocrine function, provides two hormones, glucagons and insulin, with role in regulation of sanguine glucose concentration. Secretion of these hormones can be experimental influenced by meaning of some substance administration, which produces morphological alterations of endocrine pancreas, as there are those being induced by citotoxically agents (streptozotocine), or selective stimulation of hormonal secretion: glybenclamide, glucose (Groarke,1990, Manabe et al, 1989).

MATERIALS AND METHODS

The researches were accomplished on adult hens, grouped in 4 groups: a control hen group and a hen group for each substance administrated. The biological material was represented by Leghorn hens, aged of 65 weeks, weighing between 1.7-2.1 kg, breed specialized in egg production. The hens, grown in industrial system and were raised in batteries, feed „ad libitum" with combine fodder. They also had the benefit of water at discretion and natural illumination.

Hens from control group were treated, for 10 days, with NaCl 0.9%, by intramuscularly injection of a 1 ml/kg/day dose. Streptozotocine was administrated hens of group A, in the first day of experiment, in a unique dose of 12.5 mg/kg life weight, injected intramuscularly in the peritoneal muscles. Insulin was administrated to hens of group B in a unique dose of 5 I.U./kg life weights, for 10 days, injected intramuscularly (in the peritoneal muscles). To hens of group C, glybenclamide was administrated oral, individual, in dose of 20 mg/kg/day.

The experiment was carried out for 10 days, period in which the hens were permanently clinic supervised. From two in two days, before the moment of substance administration, a drop of blood was harvested from hens crest, for glycemia test. The influence of the above-mentioned substances upon pancreatic juice flow was established by harvesting the juice flow, obtained as a background secretion trough the acute fistula of the main drain (Wirsung). The pancreatic juice samples harvested were volumetric measured for the establishing the enzymatic flow and analyzed concerning protein concentration. After 10 experimental days, all hens were subject of pancreatic juice harvest, using laparotomy and fistula of the main pancreatic drain. The quantity of pancreatic juice harvested from each hen, during one hour, was diluted with 1 ml distilled water. The pancreatic juice samples were freezing at –15^{0}C, until enzymatic evaluation of pancreatic lipase and amylase was made. After pancreatic juice harvest, blood samples were taken, obtaining from them serum (trough sedimentation and centrifugal extraction for 10 minutes at 3000 rpm). After that, the hens were sacrificed and pancreas fragments for histological study were harvested. From each group, amylase and lipase activity, from pancreatic juice and sanguine serum, were calculated. The results were statistically processed, obtaining the average and the standard error of the average (X±Sx). For statistical signification assignment, the different between groups were compared trough Student Test.

RESULTS

Because in the literature is noted that some hormones and substance can induce changes in flow and composition of pancreatic juice, the aim of our experiments was to determine the effect of streptozotocin, insulin and glibenclamide on pancreatic juice flow and on its enzymatic activity (Kato, 1989, Soling et al, 1992). Table 1 presents the mean of pancreatic juice flow, collected during one hour, from the hens from all studied groups, compared to the control group mean values.

Table 1: Influence of streptozotocin, insulin and glybenclamid on pancreatic juice flow in hens (ml/hour)

No.	Group	Pancreatic juice flow (ml/hour) X±Sx
1	Control group (n=10)	0.235± 0.045
2	A (n=10) treated with Streptozotocine	0.323± 0.058
3	B (n=10) treated with Insulin	0.172± 0.03
4	C (n=10) treated with Glybenclamide	0.285±0.057

n – hens number, X±Sx = average ± the standard error of the average; P > 0.05

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In hens from group treated with streptozotocin, the flow of pancreatic juice is increased by about 0.5 times compared to the hens from the control group, but not significant (P>0.05). The data analysis shows that in hens from the both experimental groups, treatment with glybenclamide and insulin, the pancreatic juice flow difference between the two groups and the control group was, also, statistically insignificant (P>0.05). The experimental protocol and the work method used, have allowed us to harvest a pure pancreatic juice, in enough quantity to accomplish the tests regarding enzymatic activity of amylase and lipase from pancreatic juice evaluation. The influence of streptozotocin, insulin and glybenclamide on amylase activity and total protein levels in pancreatic juice in hens are presented in Table 2.

Table 2: Influence of streptozotocin, insulin and glybenclamide on amylase and lipase activity and total protein levels in pancreatic juice in hens

No.	Group	Amylase activity and the total protein level from the pancreatic juice (X±Sx)	
1	M (n=10) control group	Amylase activity (U/dl)	14415.6±1811.1
		Lipase activity (UL/ml)	4.54±0.43
		Protein (g/dl)	2.58±0.29
2	A (n=10) treated with Streptozotocine	Amylase activity (U/dl)	14929.7±1393.6
		Lipase activity (UL/ml)	5.1±0.85
		Protein (g/dl)	2.73±0.56
3	B (n=10) treated with Insulin	Amylase activity (U/dl)	22419.4±4901.1
		Lipase activity (UL/ml)	3.48±0.51
		Protein (g/dl)	4.94±1.18
4	C (n=10) treated with Glybenclamide	Amylase activity (U/dl)	11238.3±1910.1
		Lipase activity (UL/ml)	3.28±0.78
		Protein (g/dl)	2.30±0.25

n – hens number, X±Sx = average ± the standard error of the average; P > 0.05;

U/dl –Units of lipase activity/dl, UL/ml - UL - Lipase units – ml NaOH 0,05N/37°C/18 hours)

Streptozotocine administrated in hens from group A, has determined an intensification of pancreatic amylase (14929,7±1393,6 U/dl) and lipase activity (5,1±0,85 U/dl), but statistically insignificant (P>0.05) in comparison with control group. In hen, streptozotocine, doesn't cause any meaning injury of islets and pancreatic acinus. All these results, accentuates a different behavior of β cell population from hens pancreas, comparative with mammals, at streptozotocine's actions, administrated in present study conditions (Dojana et al, 2006). Increasing the flow of pancreatic juice, while maintaining a relatively constant protein concentrations, means an increase in the overall enzymatic activity of pancreatic juice, its protein is fully enzymic. Amylase activity of pancreatic juice in hens from group treated with insulin is pronounced intensified, compared to the hens from the control group, it increased from 14415.6±1811.1 U/dl (control group) at 22419.4±4901.1 U/dl (group B). This increase in amylase activity according statistics is insignificant (P>0.05). Similarly, insulin caused an important increase in total protein content (4.94±1.18 g/dl) in the pancreatic juice in hen from group B, in comparison with control group (2.58±0.29 g/dl). However, this difference between groups are statistically insignificant (P>0.05). From the analysis of Table 2, resulted a decrease in enzyme activity of pancreatic lipase in the hens from group of treated with insulin compared to control group chickens. Lipase activity decreases is statistically insignificant (P>0.05). In hens, insulin induces a severe hypoglycemia in the first 2 hours after administration. After 3 hours, glycemia begins to recover gradual to normal values. Administrated in doses and periods of the present experiment, insulin has induced the following effects: lowering of pancreatic juice flow; increase of pancreatic amylase activity; remission of lipase activity from pancreatic juice and sanguine serum. Administrated in repeated doses, during 10 days, insulin doesn't involve structural alterations in pancreas, to attest β cell inhibitions trough a negative feedback mechanism (Dojana et al, 2000). As shown in Table 2, the pancreatic amylase activity (11238.3±1910.1 U/dl) and lipase activity (3.28±0.78 UL/ml) decreased statistically insignificant (P>0.05) in hens from the group treated with glibenclamide, compared with control group. Regarding the total protein concentration between the two groups of hens, there are no significant differences (P>0.05), while the protein concentration in hens from the group C (mean value of 2.30±0.25 g/dl) is lower than the control group (2.58±0.29 g/dl). Glybenclamide administrated in our experimental conditions, induces significant hypoglycemia in hens, which confirms his activation mechanism upon β cells. Upon exocrine pancreas, glybenclamide has produced the following effects: increase of pancreatic juice flow secretion; insignificant drop of amylase and lipase activity from pancreatic juice; remarkable effects of these enzymes upon sanguine serum. Administrated in our study conditions, glybenclamide didn't bring out any structural alterations al pancreas endocrine cell level.

CONCLUSIONS

Streptozotocine, administrated in a unique dose of 12.5 mg/kg in hens, has determined an increasing of the pancreatic juice flow (without statistically significance) and, also, an insignificant intense of pancreatic amylase and lipase activity. Administrated in doses and periods of the present experiment, insulin has induced a lowering of pancreatic juice flow, increasing of pancreatic amylase activity and remission of lipase activity from pancreatic juice and sanguine serum. Glybenclamide, individual oral administrated, in dose of 20 mg/kg/day, induced an increased pancreatic juice flow secretion, and insignificant drop of amylase and lipase activity from pancreatic juice, and also remarkable effects of these enzymes upon sanguine serum.

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