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# THE EFFECT OF THYROXINE, INSULIN, HYDROCORTISONE OR ADRENALINE ADMINISTRATION ON PANCREATIC EXOCRINE SECRETION IN RABBIT

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## ABSTRACT

The secretory response of the exocrine pancreas has been studied in rabbits submitted to thyroxine, insulin, hydrocortisone or adrenaline treatments. Pancreatic exocrine response was induced by secretine. Thyroxine and insulin treatments increased basal levels of pancreatic juice flow, protein output and amylase activity. These levels were risen several fold by the secretine stimulation. Hydrocortisone treatment decreased basal levels of pancreatic juice flow, protein output, amylase and trypsin activities, but adrenaline treatment did not. Secretine did not significantly modify the pancreatic exocrine response in hydrocortisone and adrenaline treated rabbits. In contrast, hydrocortisone and adrenaline decreased the amylase activity in the pancreatic tissue. Trypsin activity was increased in the pancreatic protein extracts from the thyroxine treated rabbits and it was decreased under the influence of the insulin treatment. The hydrocortisone and adrenaline treatments increased the serum concentrations of glucose and lipids, which is not correlated with the pancreatic exocrine response. The physiological implications of these findings are considered. It is concluded that thyroxine, insulin, hydrocortisone or adrenaline treatments modify the secretion and/or release of pancreatic enzymes in rabbits.

## INTRODUCTION

Stimulative actions on the digestive enzymatic secretion could bring economical advantages by a more efficient conversion of the feed. A number of studies were focused on the effects of diet composition on digestive enzyme secretion. Lebas *et al.* (1977) searched the relationship between digestive enzymatic equipment and the composition of feed in suckling rabbits. Dojană (1994) studied the effect of starch and vegetable oil enriched feed on pancreatic amylase and lipase activity in rabbits. Fekete *et al.* (1990) studied the effect of animal fat and vegetable oil supplementation of feeds of different energy concentration upon the digestibility of nutrients. Dojană *et al.* (1998) investigated the dynamics of main digestive enzymes in weaning period of the rabbits, when some digestive disorders may appear due to the completely replacement of the milk by solid feed. An adaptation in enzyme activity of the exocrine pancreas has been reported when the composition of diet was changed (Howard and Yudkin, 1963, Ben Abdeljlil *et al.*, 1963, Fekete *et al.*, 1990, Dojană, 1994). Other studies were focused on the effects of some hormones on the digestive enzyme secretion. Jolicoeur *et al.* (1980) published a study on the trophic effects of the hormones of gestation and lactation on the rat exocrine pancreas. An important study of the effects of some metabolic hormones on pancreatic exocrine secretion performed Harada and Kato (1982) on rats. Again Harada and Kato (1983) found that, in sheep, short-chain fatty acids act directly on pancreatic acinar cells to stimulate secretion. The present experiment was performed to study the influence of

thyroxine, insulin, hydrocortisone or adrenaline treatment on secretine induced pancreatic exocrine secretion of rabbit.

## MATERIAL AND METHODS

### *Animals and diets*

35 New Zealand White rabbits of 90 days old, with an average weight of  $1,825 \pm 33$  g were used: 7 rabbits for each hormone treated group (thyroxine, insulin, hydrocortisone and adrenaline) and 7 rabbits for a control group. The rabbits were put in individual cages, submitted to 12 hours of artificial light per day (from 8.00 a.m. to 8.00 p.m.), and had free access to water. The rabbits were fed *ad libitum* on a commercial granulated feed containing lucerne, soy-bean, wheat, barley, oats and a vitamin-mineral supplement. The feed contained (in g/kg) crude protein 155, fat 24 and crude fibre 187 and had 9.31 MJ digestible energy/kg. The control group, composed of rabbits of the same breed, age and weight, had the same environmental and feed conditions. Feed intake and body weight (b.w.) during hormone administration were noted for each rabbit group.

### *Administration of hormones*

Single doses of substance were as follow: thyroxine (Euthyrox, Merck) 50  $\mu\text{g}/100\text{g}$  b.w., insulin (Humulin Nova Regular, Lilly) 0.5 U/100 g b.w., hydrocortisone (Biofarm) 500  $\mu\text{g}/100$  g b.w., and adrenaline (Biofarm) 50  $\mu\text{g}/100$  g b.w. Thyroxine was administered *per os*, while insulin, hydrocortisone and adrenaline were injected subcutaneously. All of the doses were administered twice daily, at 12 hours intervals, for 8 days.

### *Preparation for pancreatic juice collecting*

In the ninth day, 4-5 rabbits from each group were anaesthetised with chloralose 1% in a 0.9% saline solution injected intravenously, at 38°C, in a dose of 100 mg/kg b.w. Then, the abdomen was opened, and a metal cannula (outer diameter of 0.5 mm) was inserted into the Santorini duct to collect pure pancreatic juice. A calibrated tube (1 $\mu\text{l}/\text{mm}$ ) was attached to the free end of the pancreatic duct cannula for pancreatic juice flow measurement. Then, the abdominal wall was closed with sutures. A cannula was inserted into the *vena femoralis* to inject 100 U of secretine per kg b. w. The rectal temperature of animals was maintained at 38°C by means of a heat lamp.

### *Sampling*

For ten minutes before secretine injection, pure pancreatic juice was collected, the samples being kept as basal secretion. After secretine injection, the pancreatic juice was collected for another ten minutes, the samples being kept as stimulated secretion. The samples of collected juice were diluted to a final volume of 10 ml. Immediately after measuring secretine induced pancreatic juice flow, the pancreas was rapidly excised, weighed and homogenised with sea sand in a 0.9% saline solution at 4°C, for an hour, and then centrifugated. The supernatant was considered as total proteic extract (TPE).

### *Biochemical analyses*

Amylase activity was assayed in serum, TPE of pancreas and pure pancreatic juice by the modified method of Smith and Roe (1949), using starch as substrate. Amylase activity was expressed as amylase units (AU) per mg protein. AU is the enzymatic activity which hydrolyses 1 mg of starch in 30 min at 37°C. Trypsin activity was assayed in pancreatic juice and TPE of pancreas by the method of Schwert and Takenaka (1955), using benzoyl-arginyl-ethyl-ester (BAEE) as substrate. Trypsinogen was activated using trypsin 0.5  $\mu\text{g}/\text{ml}$  of HCl solution 0.1 mmol/l. Protein concentration in TPE of pancreas and in serum was assayed by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard. Serum glucose and lipid concentrations were determined according to Manta *et al.* (1976).

*Statistical analyses*

Data were statistically processed and the results were presented as mean  $\pm$  standard error of mean. The difference of means was analysed by Student's *t* test (Tacu, 1968).

**RESULTS AND DISCUSSION**

*Feed intake and b.w.*

Figure 1 presents the average daily variation of feed intake during the eight days of hormonal treatment. The insulin treated group had an average daily feed intake 18% higher ( $P>0.05$ ) than the control group at the end of hormonal treatment period. Following 8 days of treatment, the same group showed a b. w. 4% higher than the control (Figure 2). Jolicoeur *et*

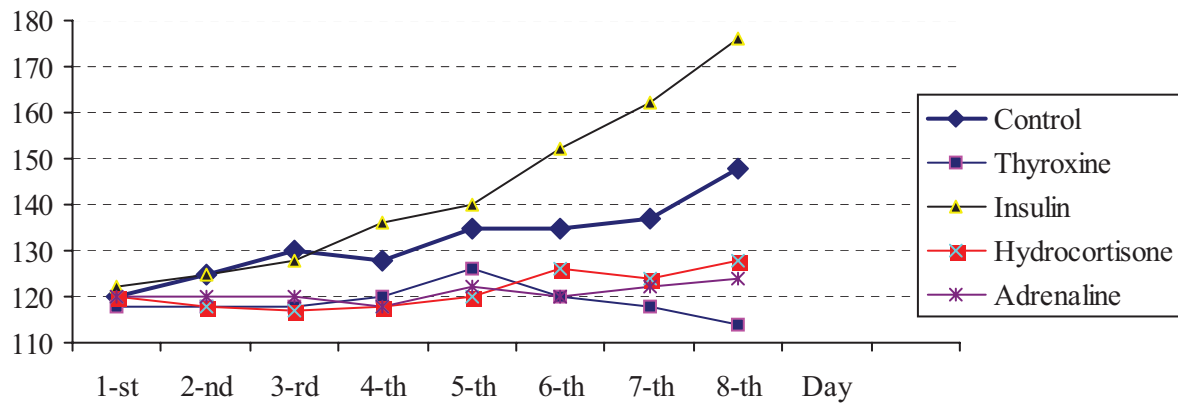


Fig. 1. Feed intake in the hormone treated groups versus control group (g/day)

*al.* (1980) reported an increase in feed consumption for nursing and pregnant rats, to be attributed to a stimulating effect of the increased prolactin secretion on the pancreas. The groups treated with thyroxine, hydrocortisone or adrenaline showed no significant decrease in feed intake, and a b.w. evolution below that of the control. In the thyroxine treated group, the decreasing of b.w. became significant ( $P<0.05$ ) after eight days of hormonal treatment. In an experiment on thyroxine treated rats, Harada and Kato (1982) obtained a b.w. evolution curve similar to this experiment. For the animals treated with thyroxine, the catabolic action of thyroxine could prevail without an increase in feed intake, leading to a decrease of the b.w.

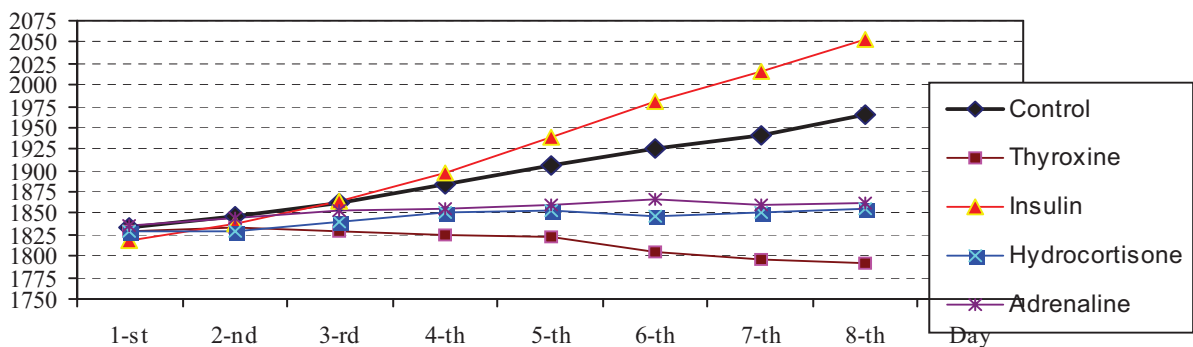


Fig. 2. Time-course of the changes in body weight of the hormone treated groups versus control group (grames)

*Pancreas exocrine secretion*

Thyroxine treatment induced a significant increase ( $P<0.01$ ) of the basal pancreatic juice flow and protein output (Table 1). For this group, the secretine stimulation increased the pancreatic juice flow about three times (in comparison to the basal flow,  $P<0.01$ ); also the protein output and amylase and trypsin activities were higher than those of the control ( $P<0.05$ ). For the insulin group, the basal

**Table 1** : Changes in pancreatic juice flow, protein output, amylase and trypsin activities evoked by secretine in hormonal treated rabbits versus control rabbits. B = basal secretion; S = secretine stimulated secretion. Each value represents the mean  $\pm$  standard error of mean of 4-5 experiments. Asterisks indicate a significant difference from the control group: \* $P<0.05$ ; \*\* $P<0.01$ .

Item	Control		Thyroxine		Insulin		Hydrocortison		Adrenaline	
	B	S	B	S	B	S	B	S	B	S
Juice flow ( $\mu\text{l}/\text{kg b.w.}/10$ min)	20.1 $\pm$ 1.8	60.8 $\pm$ 2.3	70.0 $\pm$ 12.6 **	220.6 $\pm$ 12.0 **	50.3 $\pm$ 10.0 **	180.0 $\pm$ 18.7 **	5.5 $\pm$ 0.6 *	8.5 $\pm$ 2.5	25.1 $\pm$ 5.9	27.0 $\pm$ 18.8
Protein out-put ( $\mu\text{g}/\text{kg b.w.}/10$ min)	8.8 $\pm$ 0.9	22.2 $\pm$ 3.1	15.6 $\pm$ 4.6 **	44.3 $\pm$ 3.3 *	13.0 $\pm$ 1.1 *	38.0 $\pm$ 6.9 *	3.1 $\pm$ 0.4	7.9 $\pm$ 2.6	7.6 $\pm$ 0.2	10.3 $\pm$ 2.2
Amylase ( $10^2$ AU/kg b.w./10 min)	12.2 $\pm$ 2.0	58.5 $\pm$ 2.5	24.6 $\pm$ 4.4	120.9 $\pm$ 21.3 *	26.6 $\pm$ 2.1 *	80.0 $\pm$ 18.7 *	10.0 $\pm$ 1.7	20.2 $\pm$ 3.3	16.1 $\pm$ 3.7	20.6 $\pm$ 5.6
Trypsin (nmolBAEE/ kg b.w./10 min)	12.5 $\pm$ 1.4	18.6 $\pm$ 4.2	14.1 $\pm$ 3.0	22.3 $\pm$ 3.2 *	4.0 $\pm$ 0.4	6.9 $\pm$ 0.2	10.0 $\pm$ 3.3	11.1 $\pm$ 2.1	8.3 $\pm$ 3.0	14.9 $\pm$ 9.9

pancreatic juice flow presented a significant ( $P<0.01$ ) increase accompanied by a significant ( $P<0.05$ ) increase of protein output and amylase activity, while trypsin activity was decreased. The increase of digestive enzyme activity associated with the other anabolic effects of the insulin determines the animal to gain weight. The effect of insulin on pancreatic juice flow and composition is very similar to that of thyroxine, but their effect on feed intake and daily weight are opposite. A possible explanation about the opposite effect of thyroxine versus insulin on feed intake and daily weight gain may be found in the activity of the cellular enzymes. When thyroxine is experimentally administered, intracellular synthesis of proteins, including enzyme-proteins, appears increased. Some of these enzyme-proteins act at mitochondrial level in the degradation of nutriment leading in a decrease of the b.w. (Hăulică, 1997). Secretine stimulation for the insulin group led to a three fold increase of the pancreatic juice flow ( $P<0.01$ ), together with a significant increase ( $P<0.05$ ) in the protein output and amylase activity. An insulin evoked increase of pancreatic juice flow and protein output reported Harada and Kanno (1976) in cold exposed rats. For the hydrocortisone group, the basal pancreatic juice flow showed a significant decrease from statistical point of view ( $P<0.05$ ), but there was an insignificant decrease in both protein output and amylase and trypsin activities. The significant decrease in basal pancreatic juice flow associated with an insignificant reduction effect in protein (enzyme) release, can be linked to a vasoactive effect of hydrocortisone on pancreatic arterioles. Secretine stimulated pancreatic juice secretion for the hydrocortisone group was lower versus control, both for juice flow and protein release and enzymatic activities. The hydrocortisone inhibiting effect on protein release in the pancreatic juice obtained in this experiment on rabbits matches the results reported by Harada and Kato (1982) on rats, but contradicts the effects obtained by Morisset and Jolicœur (1980), also on rats.

*Protein content and digestive enzymatic activity of the pancreas tissue*

Except for the thyroxine group, in which the pancreas protein content decreased significantly ( $P<0.05$ ) versus control group, for the groups otherwise hormonally treated, the protein content was not significantly altered versus control group (Table 2).

**Table 2** : Protein content and amylase and trypsin activities in TPE of the pancreas in the control rabbit group and each hormone treated rabbit group. Numbers in parentheses indicate number of animals. \* $P<0.05$ ; \*\* $P<0.001$ ; \*\*\* $P<0.0001$

Group	Protein (mg/g pancreatic tissue)	Amylase activity (AU/mg protein)	Trypsin activity (nmol BAEE/mg protein)
Control (7)	245 ± 12	568.0 ± 28.7	1.310 ± 0.014
Thyroxine (7)	140 ± 26*	608.1 ± 48.2	3.885 ± 0.024***
Insulin (7)	230 ± 21	526.7 ± 98.2	0.824 ± 0.022*
Hydrocortisone (7)	220 ± 10	326.7 ± 20.4*	1.320 ± 0.005
Adrenaline (7)	210 ± 9	108.0 ± 14.4***	1.500 ± 0.032

The amylase content of the pancreas TPE decreased significantly for the groups treated with hydrocortisone (43%,  $P<0.05$ ), and adrenaline (81%,  $P<0.001$ ), but not for the thyroxine and insulin groups. Our data concerning the decreased amylase activity of the TPE for the hydrocortisone and adrenaline groups are according with the data obtained by Harada and Kato (1982) on rats. However, these authors found the same decrease of pancreas amylase content in rats treated with thyroxine. On the other hand, trypsin activity increased significantly ( $P<0.001$ ) in the thyroxine group, and decreased in the insulin and adrenaline groups. The serum amylase activity, glucose and lipid concentrations are presented in Table 3.

**Table 3** : Serum amylase and glucose and lipid concentrations in each hormone treated rabbit group, comparing with the control group. Numbers in parentheses indicate number of animals. \* $P<0.05$

Group	Serum protein (g/dl)	Amylase (UA/g of serum protein)	Glucose (mg/dl)	Lipids (mg/dl)
Control (7)	8.28 ± 0.56	68.4 ± 0.1	122 ± 12	660 ± 32
Thyroxine (7)	8.04 ± 0.87	53.1 ± 12.1	78 ± 9*	598 ± 21*
Insulin (7)	9.29 ± 0.63	80.3 ± 7.9	76 ± 18*	620 ± 43
Hydrocortisone (7)	9.23 ± 1.12	37.3 ± 0.3	138 ± 32	687 ± 24
Adrenaline (7)	8.85 ± 0.44	31.8 ± 5.8	132 ± 21	698 ± 20*

The serum amylase activity increased for the insulin groups, the amount of amylase reaching the blood being proportional to that synthesized by the pancreas (Dojană, 1994). The blood glucose concentration decreased for the groups treated with thyroxine (64%,  $P<0.05$ ) and insulin (62%,  $P<0.05$ ), and increased in the groups treated with hydrocortisone (113%,  $P<0.05$ ) and adrenaline (108%,  $P<0.05$ ). Hydrocortisone intensifies hepatic gluconeogenesis and inhibits insulin effects on glucose uptake into the muscle and liver tissues, leading to an increase of blood glucose concentration. Adrenaline also increases blood glucose concentration, both directly, by the glycogen mobilization, and indirectly, by stimulating the glucagon secretion and inhibiting the insulin secretion, for the maintenance of fuel homeostasis. Serum lipid concentration increased significantly for the adrenaline group, probable as a result of adrenaline stimulating effect on the lipase within the fat tissue.



In conclusion, based on the results of the present experiment, we suggest that thyroxine, insulin, hydrocortisone and adrenaline modify the secretion and/or release of pancreatic enzymes in rabbits, which could be of economical interest.

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