EFFECTS OF EXOGENOUSLY ADDED SHORT-CHAIN FATTY ACIDS ON PANCREATIC EXOCRINE SECRETION IN DOMESTIC RABBIT

DOJANA N[1]., POP A[2] ., PAPUC C[3].

[1]Department of Animal Physiology, Faculty of Veterinary Medicine, Str. Splaiul Independentei no.105, Bucharest, 76201, Romania.
dojanan@yahoo.com
[2;3] Department of Biochemistry, Faculty of Veterinary Medicine, Bucharest, Romania

ABSTRACT

A number of 26 New Zealand White rabbits were anesthetized and surgically prepared by acute fistula of Santorini duct to collect pure pancreatic juice. Acetate, propionate or butyrate and/or secretin were intravenously injected to search their effect on pancreatic juice flow and composition. It was fount that ntravenous administration of propionate or butyrate significantly potentiated the stimulatory effect of secretin on pancreatic juice flow. Maximal increasing of juice flow was obtained when it was administered an intravenous dose of 640 µMols of acetate, propionate or butyrate + 2 U of secretin/Kg body weight. The highest stimulatory effects on pancreatic juice flow, protein output and amylase output showed butyrate + secretin, following propionate + secretin.

Key words: short-chain fatty acids, secretin, pancreatic juice.

INTRODUCTION

Despite of the large number of the works in the field of digestive physiology of the domestic rabbit, unknowns persist. It is well-known that diet components such us fats, starch or proteins stimulate the secretion of digestive enzymes, but the exact effect of nutritional components remains still unknown (DOJANA *et al.*, 2000; FERNÁNDEZ-CARMONA *et al.*, 2000, HARADA *et al*., 1999, KATOH *et* TSUDA, 1984). Short-chain fatty acids, which are the major products of microbial fermentation in the alimental canal of herbivores, directly stimulate pancreatic endocrine and exocrine secretions. However, there are differences among species in the response induced by short-chain fatty acids: exocrine pancreas of sheep, guinea pig, and voles respond to these fatty acids, but those of mice, cats, and fowls do not (HARADA *et al.* 1999) The aim of this study was to find the effects of the main short-chain fatty acids (acetate, propionate and butyrate) on the flow and composition of pancreatic juice in domestic rabbit.

MATERIAL AND METHODS

Animals and diets

A number of 26 New Zealand White rabbits of six months old, with an average weight of 2.525±0.121 Kg were used in this experiment. The rabbits were hosted in common group 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 fresh water. The rabbits were fed *ad libitum* using a commercial granulated feed containing lucerne, soy-bean, wheat, barley, oats and a vitamin-mineral supplement. The feed had 7.89 MJ digestible energy/Kg. Feed was withheld for 24 H before experimentation.

Pancreatic juice collecting

The rabbits were anesthetized using chloralose 1% in saline solution, 0.1 g/Kg body weight (b.w.). Than, the abdominal wall was opened and the Santorini duct was identified and connected to a silver cannula (1.5 mm outer diameter) to collect pure pancreatic juice. The pylorus was ligatured in all experiments. The abdominal wall was closed using interrupted sutures. For intravenous injections, a cannula was inserted into the *vena femuralis dextra*. The rectal temperature of animals was maintained at 38°C using a heat lamp. The collected pure pancreatic juice was volumetric measured using a calibrated transparent tube (10 µL/cm), which was attached to the silver cannula.

Drugs

Acetate, propionate and butyrate (Biofarm, Bucharest) micromolar (µM) solutions were prepared using distilled water. The pH of the solutions was adjusted to 7.0 value with a sodium hydroxide solution. Porcine secretin (Eisai, Tokyo) was also used.

Procedure and sampling

First, secretin (2 U/Kg b.w.), acetate, propionate or butyrate (200 µMols/Kg b.w. each one) were injected and the pure pancreatic juice was collected for 30 minutes. Second, secretin (2 U/Kg) was injected simultaneously with 200 µMols/Kg acetate, propionate or butyrate and the pancreatic juice was again collected for another 30 minutes. For comparing, the pancreatic juice was collected for 30 minutes from an identically prepared control group rabbits, which were injected with the same volume of saline solution. Third, secretin (2 U/Kg b.w.) and increasing doses (10, 20, 40, 80, 160, 320, 640 and 1,280 µM/Kg b.w.) of acetate, propionate or butyrate were intravenously injected and pancreatic juice was again collected for 30 minutes. A period of five minutes before any fatty acids or secretin injection, pure pancreatic juice was collected, the samples being kept as “basal” secretion. After fatty acid and/or secretin injection, the pancreatic juice was measured from 5 to 5 minutes, the samples being kept as “stimulated” secretion. The samples of collected juice were diluted to a final volume of 10 ml and kept at –15°C before analysis.

Biochemical analysis

It was determined the total content of protein and the activity of amylase in all the pancreatic juice samples. Protein concentration in pancreatic juice was determined by the method of LOWRY *et al.* (1951), using bovine serum albumin as a standard. Amylase activity was assayed by the modified method of SMITH and ROE (1949), using starch as substrate. Amylase activity was expressed as amylase units (AU) *per* mg protein of pancreatic juice. AU is the enzymatic activity, which hydrolyses 1 mg of starch in 30 min, at 37°C and pH 7.0.

Statistical analysis

Data were statistically analyzed and the results were presented as means ± standard error of mean. The significance of mean difference between control and experimental groups was analyzed by Student's *t* test.

RESULTS AND DISCUSSION

In Figure 1 it is showed the effect of intravenous injection of 0.2 U/Kg b.w. of secretin or 200 µMols/kg b.w. of acetate, propionate, butyrate, or the same doses of secretin + acetate, secretin + propionate or secretin + butyrate. No significant differences of total amount of collected pancreatic juice were found between control and short-chain fatty acid injected rabbits ($P > 0.05$). The juice flow induced by secretin alone increased and reached a peak value of 17 µL/5 min./Kg b.w. in 10 minutes from injection, with a total amount of 58 µL of collected pancreatic juice in 30 minutes from injection. When both 200 µM of acetate, propionate or butyrate and 2 U/Kg b.w. of secretin were administered, the peak values were (in µL/Kg b.w./5 minutes): 15, 21 and 28, respectively and the total amounts of collected pancreatic juice were: 50.0 for acetate + secretin, 80.8 for propionate + secretin and 106.0 for butyrate + secretin, respectively. The peak value of butyrate + secretin was significantly higher ($P<0.01$) than secretin alone.
Pancreatic juice flow reached the peak values in 10 minutes from the intravenous injection, than began to decrease reaching basal levels in the next 20 minutes.

In this experiment it was showed that propionate or butyrate alone did not stimulate the exocrine secretion of the pancreas but they potentiated the stimulatory effect of secretin. In regard to this potency of short-chain fatty acids on pancreatic exocrine secretion, HARADA *et al*. (1999) found using the isolated, perfused pancreatic acini of guinea pigs, a similar situation: continuous administration of 100 □M propionate failed to significantly increase amylase release. The response to a combination of carbachol (10 □M) and propionate (100 □M) was significantly greater than the summated value obtained with carbachol or propionate alone. The exact mechanism of this release is still unknown.

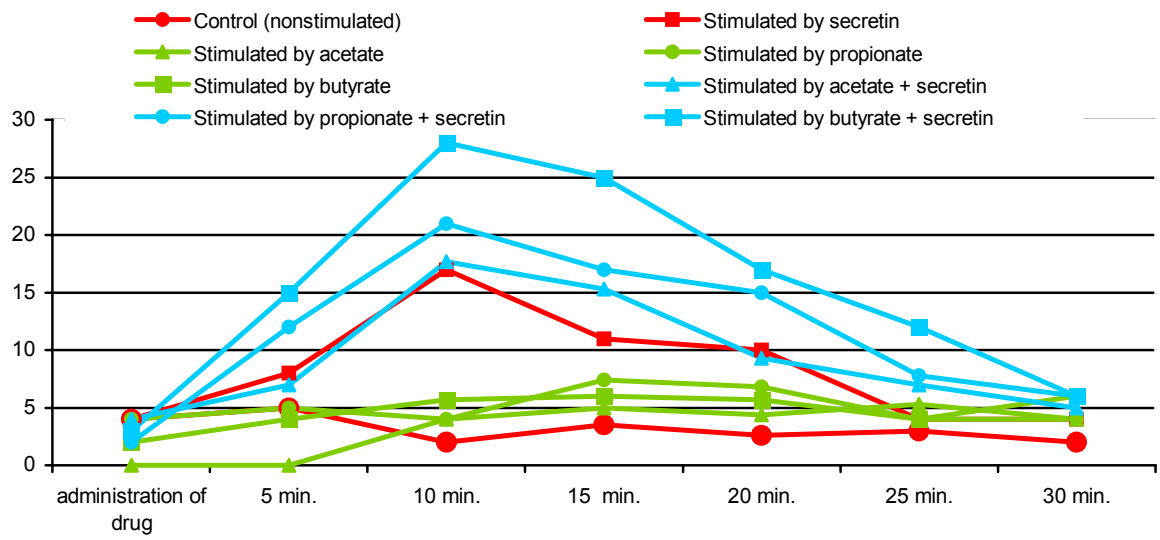

Figure 1. Time course of the pancreatic juice flow in anesthetized rabbits with acute fistula of Santorini duct, following intravenous injection of 0.2 U/Kg b.w. of secretin or 200 µMols/kg b.w. of acetate, propionate, butyrate, or the same doses of secretin + acetate, secretin + propionate or secretin + butyrate (each value represents the mean of 3 - 4 experiments)

The effect of increasing doses of acetate, propionate or butyrate on the total amount of pancreatic juice flow is presented in Figure 2. The minimal dose, which significantly stimulated the secretion of pancreatic juice was 20 µMols for butyrate. Higher doses of propionate and acetate were needed to significantly increase the pancreatic juice flow: 40 and 80 µMols/kg b.w., respectively ($P<0.05$ when compared with basal secretions). Maximal doses, which induced an increasing of juice flow was 640 µMols for all the three studied short-chain fatty acids. The total amounts of juice flow following 30 minutes after 640 µMols of butyrate, propionate or acetate injections were: 123 µL/Kg b.w. for butyrate, 110 µL/Kg b.w. for propionate and 48 µL/Kg b.w. for acetate.

Figure 3 shows the time course of pancreatic juice flow, amylase output and total protein output, which were stimulated by 640µMols/Kg b.w. of acetate, propionate, or butyrate + 2 U/kg b.w. of secretin. The pancreatic juice flow peak of butyrate was significantly higher than that of acetate ($P<0.01$). Amylase output rapidly increased and reached the peak 10 minutes following the intravenous injection for all the three short-chain fatty acids. The highest amylase output showed the butyrate injected rabbits, with a peak of 1,150 AU/5 min/kg b.w. The total amount of released protein following 30 minutes of collecting juice was highest in butyrate injected rabbits: 4.63 µg/kg b.w./5 min. The quantity of protein released by butyrate was significantly greater ($P<0.05$) than that of acetate. Our results on rabbits are in accord with those of KATOH *et* TSUDA (1984) on sheep, who found that the pancreatic juice release, evoked by short-chain fatty acids, increases with the increasing of carbon number of the short-chain fatty acid molecule.

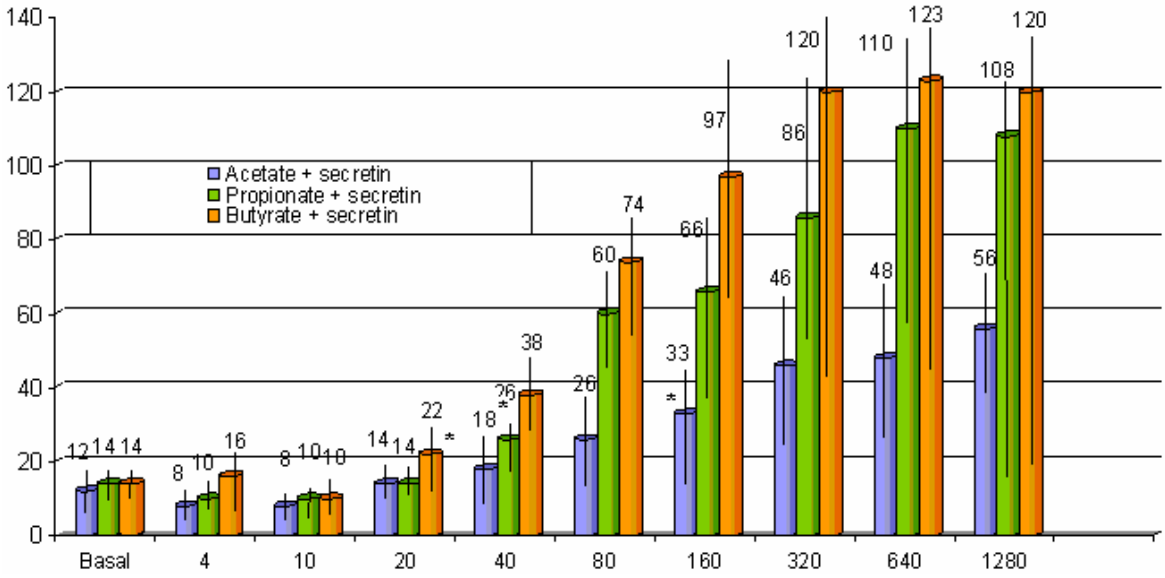

Figure 2. The total amounts of pancreatic juice flow (in µL/Kg b.w.) obtained from domestic rabbits with acute fistula of Santorini duct, following 30 minutes from intravenous injection of 2 U/kg b.w. secretin and various doses (in µM/kg b.w.) of acetate, propionate or butyrate. Each value represents mean ± standard error of mean of 4 – 6 experiments

PARIENTE *et al.* made an experiment on the effects of H1 and H2 receptor agonists and antagonists on rabbit pancreatic exocrine secretion stimulated by secretin and cholecystokinin. Their results suggest that H1 receptors have stimulatory effects, while H2 receptors have inhibitory effects on exocrine rabbit pancreas. It is possible an experimental effect could be influenced by the ratio of H1 and H2 receptors on pancreatic acinar cells. This fact could explain the variety of results in experimental stimulating of pancreatic juice secretion in rabbit, using a variety of final digestion products: amino acids, volatile fatty acids, long-chain fatty acids etc. (HARADA *et al*. 1999; HARADA *et* KATO, 1983; PARIENTE *et al*., 1999).

Even it was seen that short-chain fatty acids potentiate the stimulatory effect of secretin on the pancreatic juice secretion in rabbit, the exact mechanism remains to be explained. We don't know whether, in rabbit, the volatile fatty acids directly stimulate the secretin secretory cells, or they act on pancreatic acinar cells, as in sheep (HARADA *et* KATO, 1983), or they act *via* autonomic nervous system.

CONCLUSION

Butyrate, propionate and acetate enhance the stimulatory effect of secretin on pancreatic juice flow, protein output and amylase output in anesthetized rabbits. Butyrate seems to be the most potent, following propionate.

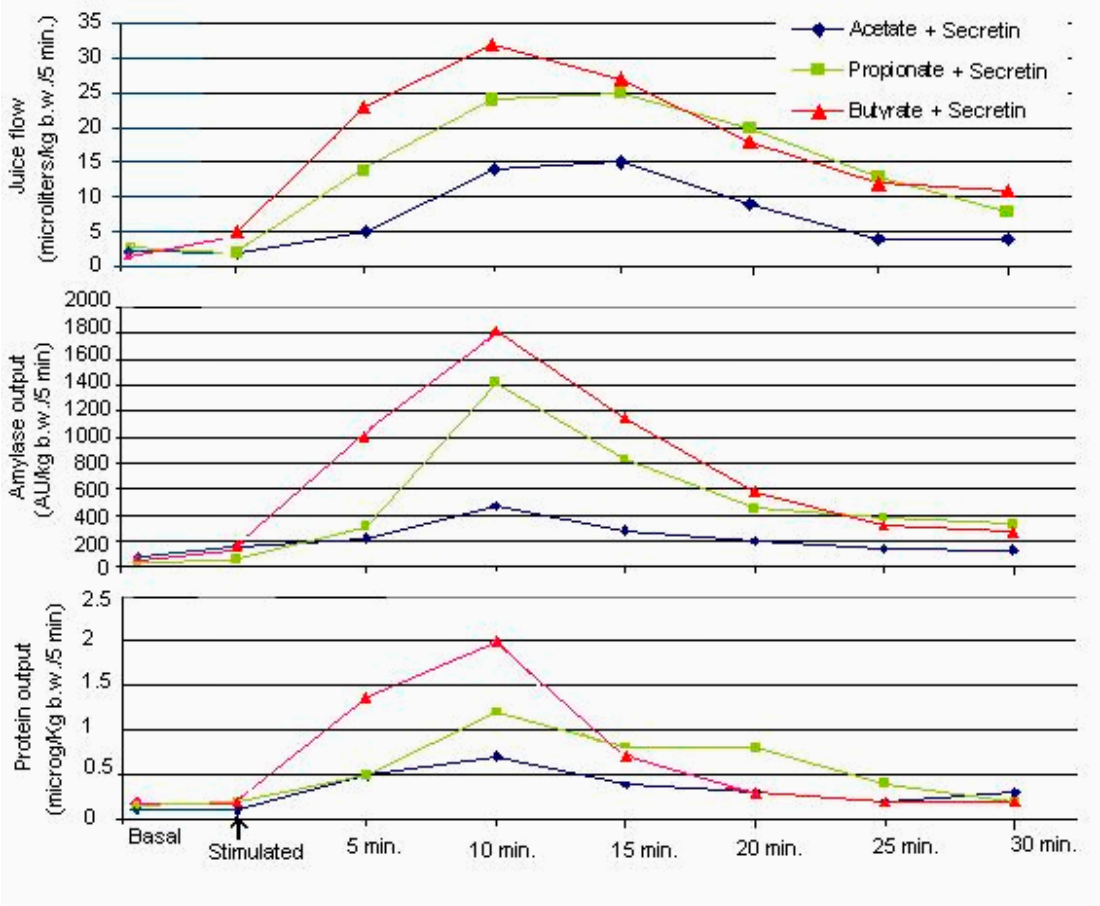

Fig. 3. Time course of pancreatic juice flow, protein and amylase output induced by intravenous administration of 640 µMols/kg b.w. of acetate, propionate or butyrate simultaneously with 2 U of secretin in domestic rabbits with acute fistula of Santorini duct (each value represents the mean of 3 - 4 experiments)

REFERENCES

DOJANA N., DINISCHIOTU A., MILITARU M. 2000. The effect of thyroxine, insulin, hydrocortisone or adrenaline administration on pancreatic exocrine secretion in rabbit. *Proceedings 7th World Rabbit Congress. Valencia*, C vol.: 275-182.

FERNÁNDEZ-CARMONA J., PASCUAL J. J., CERVERA C. 2000. The use of fat in rabbit diet. *Proceedings 7th World Rabbit Congress. Valencia*, **1**:217-222.

HARADA E., KATO S. 1983. Effect of short-chain fatty acids on the secretory response of the ovine exocrine pancreas. *Am. J. Phys*, **244**: G284-G290.

HARADA E., MITANI M., TAKEUCHI T. 1999. Potentiation of carbachol-induced amylase release by propionate in guinea pig and vole pancreatic acini. *Am. J. of Physiol. Regal. Integr. Comp. Physiol*. **277**: R767-R775.

KATOH K., TSUDA T. 1984. Effects of acetylcholine and short-chain fatty acids on acinar cells of the exocrine pancreas in sheep. *J. of Phys.* **356**:479-489.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L., RANDALL R. J. 1951. Protein measurement with the folin phenol reagent. *J. of Biol. Chem.* **193**: 265-275.

PARIENTE J. A., MADRID J. A., SALIDO G. M. 1990. Role of histamine receptors in rabbit pancreatic exocrine secretion stimulated by cholecystokinin and secretin. *Exp. Physiol.* **75**(5): 657-67.

SMITH B. W., ROE J. H. 1949. Photometric method for the determination of α-amylase in blood and urine. *J. Biol. Chem.,* **179**: 53-59.