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ANIMAL HUSBANDRY

The Role of Nitric Oxide in the Exocrine Pancreatic Function in Chicken

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Abstract—The universal cell mediator nitric oxide (NO) mediating the physiological effects via so-called NO donor compounds prolonging its life time. The concentration of NO metabolites in the pancreatic juice of chicken was determined by the enzymatic sensor. It was found that pancreatic juice from starved birds contains nitrate and no other nitro- or nitroso compounds. After feeding, the NO donors appear in pancreatic juice in concentrations of a few tens of μM. These compounds were present in pancreatic juice for several hours after feeding. It is possible that NO donors promote relaxation of the smooth muscles in the walls of pancreatic ducts. Concentration of NO donors in blood serum also increased 2.5–3.0-fold after feeding and remained high during a few subsequent hours. The preliminary subcutaneous administration of atropine to the birds prevent this increase with no effects on the initial concentrations of NO donors in serum. This suggests that the increase is associated with cholinergic stimulation and, therefore, with the activity of the parasympathetic nervous system. The concentration of trypsin in serum also increased after feeding, and atropine also prevents this increase. It is known that serum trypsin acts as a regulator of pancreatic function. Intravenous administration of trypsin led to an insignificant increase of tryptic activity in serum but induced the increase of NO donor concentration twofold. Apparently, nitric oxide, represented by these compounds, can act as a humoral factor in the regulation of pancreatic function in pancreatic juice and possibly in the blood.

Keywords: chicken, pancreas, nitric oxide (NO), nitrite, nitrate, trypsin

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INTRODUCTION

Regulation of pancreatic activity has nervous and humoral components. It is known that the parasympathetic mediator acetylcholine provides physiological effects through the universal cellular mediator nitric oxide (NO) by the activation of NO synthase [1]. Nitric oxide is a short-lived substance synthesized from arginine by the action of the enzyme NO-synthase. NO rapidly oxidizes to nitrite and nitrate. Nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC's), and high-molecular nitro compounds capable of transforming to DNIC's (RNO_2) are also formed in living tissues. There is an assumption that all these compounds protect NO from oxidation to toxic nitrite, prolong its physiological lifetime, and directly interact with NO targets [2]. The rapid determination of the entire spectrum of nitric oxide metabolites in biological objects raises a technical difficulty due to their diversity, the relatively low selectivity of modern techniques [3, 4]. But it is impossible without this to quantify the effects of endogenously synthesized NO, and, therefore, put forward some hypotheses about its specific physiological role and mechanism of action. The findings on the mediator role of nitric oxide in the regulation of pancreas function were made on the basis of experiments with NO synthase blockers [5–7]. However, it is not possible to answer the question of how much endogenous NO is synthesized and at what particular stage it has an effect.

An enzymatic sensor developed at the Research Center of Pirogov of the Russian National Research Medical University together with the Institute of Chemical Physics of the Russian Academy of Sciences makes it possible to quickly determine the total content of nitro- and nitroso compounds as well as the main physiologically significant groups of NO metabolites in various media [4, 8].

The purpose of this work is to determine the dynamics of the content of endogenously synthesized NO and its correlation with the activity of pancreatic

enzymes in secrete of pancreas and blood before and after feeding.

MATERIALS AND METHODS

The experiments were carried out on Hisex white breed 9–10-month-old chickens. The birds were kept in the vivarium of the All-Russia Research and Technological Institute of the Poultry of the Russian Academy of Sciences, fed in accordance with zootechnical norms [9]. After a 16-h feed deprivation, 2–3 mL of blood was taken from an axillary vein. A 3.8% sodium citrate solution was used as an anticoagulant. The selection of the pancreatic secret was carried out according to the previously described method [10].

The activity of enzymes in the pancreatic juice due to their high activity was determined by classical methods: amylases by starch hydrolysis and trypsin by casein hydrolysis [11]. The activity was expressed in milligrams of the degraded substrate per 1 mL of juice per minute. The activity of trypsin in plasma was studied by the kinetic method using nitroanilide benzoyl DL-arginine (BAPNA) as a substrate on semiautomatic biochemical analyzer BS3000P (China) [12].

Plasma amylase and lipase activity was determined by the biochemical automatic analyzer Chemwell2900(T) (United States) using the corresponding Human reagent kits (Germany), and the amount of hydrolyzed substrate (μM) in 1 liter for 1 min was expressed in U/L.

An enzyme sensor [8] was used to determine the content of NO donors and nitrate. It based on the unique ability of all nitroso compounds to inhibit catalase in the presence of halide ions with approximately equal efficiency. Other known catalase inhibitors do not have this feature and normally are not found in bio objects in concentrations that can introduce artifacts. Dinitrosyl iron complexes containing thiolate ligands (DNIC/SH) lose their inhibiting ability in a medium containing an iron chelator (o-phenanthroline, EDTA) and a NO trap (hemoglobin) intercepting nitric oxide released from the complex. S-nitrosothiols (RSNO) were defined as compounds transforming into DNIC/SH under the influence of ferrous iron and thiols and acquiring their properties. Nitrite (NO_2^-) and nitrosoamines (RNNO) hardly produce DNIC/SH in a neutral medium and retain inhibitory abilities with the sequential addition of ferrous iron, glutathione, NO trap, and iron chelator. Iron-containing nitrosyl complexes that do not contain thiols (Fe- (NO) n) were defined as compounds that acquire properties (DNIC/SH) after adding glutathione to the reaction medium. The existence of such compounds has been shown in previous works [4]. High molecular nitrates (RNO_2) were defined as compounds that acquire the inhibitory properties of DNIC/SH under the influence of ferrous iron and glutathione [8].

To determine the total pool of nitro compounds, the ability of 3-chloride vanadium to recover them to the nitroso state and acquire the ability to inhibit catalase was used. Catalase activity was determined by calorimetry. The method does not require any preliminary sample preparation. The sensitivity of the method is 40 nM [8].

For intravenous administration, a solution of crystalline trypsin preparation in sterile saline was used. The trypsin activity, determined by the kinetic method [12], was 291 ± 15.1 U/L, and 5 mL solution was injected into the axillary vein. Blood was taken and the enzyme activity and the content of NO donors were determined after 1 h.

To assess the effect of the parasympathetic nervous system, atropine sulfate was injected subcutaneously in a dose of 1.7 mg/kg chicken body weight 1 h before feeding of the experimental group.

Statistical processing of results was performed using an Excel computer program, the accuracy of the results was determined using Student's tables, and the difference was considered reliable at $P < 0.05$.

RESULTS AND DISCUSSION

After a 16-h break in feeding, pancreatic juice hardly contained any NO donor compounds. However, 0.5 h after the start of feeding, a substance, defined as Fe (NO)n, at a concentration of up to 25 μM was detected in it. Since such complexes do not contain glutathione, cysteine, they can also exist in an environment rich in proteolytic enzymes. They are not detected by the EPR method used for the detection of nitrosyl iron complexes [4]. No other NO donor compounds were found in the pancreatic juice (Table 1). After 1 h, the concentration of Fe (NO)n decreased to 10 μM, while that after 2.5 h decreased to 0.6 μM.

An increase in the concentration of NO donors in the pancreatic juice 0.5 h after feeding occurred simultaneously with an increase in the activity of pancreatic enzymes: trypsin, amylase, and lipase. The activity of enzymes continued to remain high even 2.5 h after the start of feeding, and the content of NO donors decreased to a minimum (Table 1). Nitric oxide is known to have a relaxing effect on smooth muscle. NO donors contained in the juice probably stimulate relaxation of the smooth muscles of the gland ducts. A number of studies have shown that intravenous administration of NO-synthase blockers caused a violation of duodenal motility and secretion of pancreatic juice [7].

In the blood, prior to feeding, the content of Fe(NO)n is 10 μM, that after 0.5 h is 28 μM, and it decreases to 15 μM after 3 h (Table 2). What caused the increase in the concentration of NO donors? It should be noted that the activity of trypsin also increased with a rise of NO donor concentration but

Table 1. Content of NO donors and the activity of digestive enzymes in pancreatic secret ($n = 20$)

Period	Fe(NO)n content, μM	Trypsin activity, mg/(mL min)	Amylase activity, mg/(mL min)	Lipase activity, U/L
Before feeding	<0.1	90 ± 13.3	1955 ± 334.1	3865 ± 430.1
After, hh.:				
0.5	22.2 ± 2.5	230 ± 27.9	4244 ± 314.4	8524 ± 735.1
1.0	10.1 ± 1.8	285 ± 28.4	4844 ± 420.3	9171 ± 826.3
1.5	4.9 ± 1.5	313 ± 28.5	5183 ± 673.0	8293 ± 925.7
2.0	2.1 ± 1.2	310 ± 29.6	5294 ± 630.1	10096 ± 973.5
2.5	0.6 ± 0.4	309 ± 34.2	5411 ± 503.3	7887 ± 904.3

In all samples, the content of NO_2^- + RNNO, RSNO, DNIC/SH, and RNO_2 < 0.1 μM, nitrate concentration is 40–60 μM.

Table 2. Digestive enzyme activity and content of Fe(NO)n in the blood of chickens

Period	Fe(NO)n content, μM	Trypsin activity, U/L	Amylase activity, U/L	Lipase activity, U/L
Before feeding				
After, hh.:	10.1 ± 2.8	75.2 ± 2.3	281.3 ± 45.1	14.1 ± 0.8
0.5	28.7 ± 2.1	134.3 ± 10.8	336.1 ± 49.2	12.2 ± 0.6
1.0	18.6 ± 1.93	129.1 ± 12.3	278.6 ± 19.1	14.4 ± 1.3
3.0	14.9 ± 2.8	96.2 ± 8.9	299.2 ± 20.2	13.1 ± 0.9

Difference between indices of before and after feeding is $P < 0.05$. In all samples, the content of NO_2^- + RNNO, RSNO, DNIC/SH, and RNO_2 < 0.1 μM, nitrate concentration is 50–70 μM.

Table 3. Effect of atropine and trypsin on the activity of digestive enzymes and the content of Fe (NO)n in the blood of male chickens ($n = 15$)

Period	Fe(NO)n content, μM	Trypsin activity, U/L	Amylase activity, U/L	Lipase activity,U/L
		Control		
Before feeding	11.3 ± 2.5	88 ± 6.5	281 ± 28.4	20 ± 1.6
After an hh.	19.8 ± 2.7	141 ± 11.4	245 ± 29.6	16 ± 2.0
		+ atropine		
Before feeding	10.8 ± 2.6	195 ± 26.1	203 ± 21.8	19 ± 1.4
After an hh.	10.7 ± 2.9	184 ± 18.1	203 ± 24.1	21 ± 1.1
		+ trypsin		
Before injection of trypsin	9.4 ± 1.5	229 ± 26.6	133 ± 11.6	20 ± 3.4
After an hh.	20.6 ± 1.9	244 ± 15.5	131 ± 7.4	16 ± 1.7

the activity of amylase and lipase has not changed significantly.

The introduction of atropine prevented an increase in the content of Fe(NO)n in the blood but did not affect its initial level. Atropine also prevented an increase in the activity of enzymes (Table 3). Consequently, an increase in the Fe(NO)n content, as well as an increase in the activity of enzymes in the blood after the start of feeding, is associated with the activation of the parasympathetic nervous system, which provides a complex-reflex phase of pancreatic secretion regulation.

A number of studies have shown that the activity of trypsin in the blood has a regulatory effect on the function of the pancreas [10, 13]. The injection of trypsin into the blood caused a significant increase in the content of NO donors (Table 3). This suggests a connection between the activity of trypsin in the blood and the concentration of NO donors.

Thus, intensive synthesis of nitric oxide upon activation of the exocrine pancreatic activity is due to cholinergic stimulation. Apparently, NO also acts as a humoral factor regulating pancreatic function, both in pancreatic secretions and, possibly, in the blood.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Moncada, S., Palmer, R., and Higgs, E., Nitric oxide: Physiology, pathophysiology, and pharmacology, *Pharm. Rev.,* 1991, vol. 43, no. 2, pp. 109–142.
2. Vanin, A., Dinitrosyl iron complexes with thiolate ligands: Physico-chemistry, biochemistry and physiology, *Nitric Oxide,* 2009, vol. 21, pp. 1–13.
3. Tarpey, M., Wink, D., and Grisham, M., Methods for detection of reactive metabolites of oxygen and nitrogen: In vitro and in vivo considerations, *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* 2004, vol. 286, pp. 431–444.
4. Titov, V.Y., Kosenko, O.V., Starkova, E.S., Kondratov, G.V., Borkhunova, E.N., Petrov, V.A., and Osipov, A.N., Enzymatic sensor detects some forms of nitric oxide donors undetectable by other methods in living tissues, *Bull. Exp. Biol. Med.,* 2016, vol. 162, no. 1, pp. 107–110.
5. Jyotheeswaran, S., Li, P., Chang, T., and Chey, W., Endogenous nitric oxide mediates pancreatic exocrine secretion stimulated by secretin and cholecystokinin in rats, *Pancreas,* 2000, vol. 20, no. 4, pp. 401–407.
6. DiMagno, M., Hao, Y., Tsunoda, Y., Williams, J., and Owyang, C., Secretagogue-stimulated pancreatic secretion is differentially regulated by constitutive NOS isoforms in mice, *Am. J. Physiol. Gastrointest. Liver Physiol.,* 2004, vol. 286, no. 3, pp. 428–436.
7. Onaga, T., Nagashima, C., and Sakata, T., Effect of nitric oxide synthase inhibitors on the temporal coordination of duodenal contractions and pancreatic exocrine secretion in sheep, *J. Comp. Physiol.,* 2000, vol. 170, nos. 5–6, pp. 469–479.
8. Titov, V.Yu., The enzymatic technologies open new possibilities for studying nitric oxide (NO) metabolism in living systems, *Curr. Enzyme Inhib.,* 2011, vol. 7, no. 1, pp. 56–70.
9. Fisinin, V.I., Egorov, I.A., and Lenkova, T.N., *Rukovodstvo po optimizatsii retseptov kombikormov dlya sel'skokhozyaistvennoi ptitsy* (Guidelines for Optimizing Compound Feed Recipes for Poultry), Sergiev Posad, 2014, pp. 3–4.
10. Vertiprakhov, V. and Egorov, I., The influence of feed intake and conditioned reflex on exocrine pancreatic function in broiler chicks, *Open J. Anim. Sci.,* 2016, vol. 6, pp. 298–303.
11. Vertiprakhov, V.G., Features of the secretory function of the pancreas of broiler chickens and the possibilities of correcting the digestion of animals by zeolite-based enzyme preparations, *Doctoral (Biol.) Dissertation,* Novosibirsk, 2004, pp. 59–60.
12. Mikhailova, A.G., Khairullin, R.F., Rumsh, L.D., Demidyuk, I.V., Kostrov, S.V., Grinberg, N.V., Burova, T.V., and Grinberg, V.Y., Cloning, sequencing, expression, and characterization of thermostability of oligopeptidase B from *Serratia proteamaculans*, a novel psychrophilic protease, *Protein Expression Purif.,* 2014, vol. 93, pp. 63–76. doi 10.1016/j.pep.2013.10.011
13. Laporte, J. and Tremolieres, J., Regulation hormonale de la sécrétion enzymatique du pancréas exocrine, *C. R. Acad. Sci., Ser. D,* 1971, vol. 273, pp. 1205–1207.

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