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Effects of nitric oxide associated with expression of some genes at embryonic stage of avian development

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Abstract. Data obtained by various researchers indicate the effect of nitric oxide (NO) on expression of some genes, in particular, the genes associated with myogenesis in birds. But until now it has not been possible to quantify this effect and possibility of its use, since the control of content of NO metabolites in tissues presented a methodological difficulty. The purpose of this research was to find out relationship between the NO content in tissues of avian embryos and the expression of genes responsible for myogenesis at various stages of embryogenesis. We used a highly sensitive and highly specific enzyme sensor to determine the content of the main NO metabolites in tissues. Gene expression was determined by PCR-RT. The relationship between the NO content in tissues and the expression of 7 genes involved in the process of myogenesis was studied in chicken and quail embryos. There are the genes of myocyte proliferation factor 2c (mef 2c), myogenic differentiation 1 (MyoD1), myogenesis factor 5 (myf 5), myosin (mhy 1), myogenin (myog), somatostatin (MSTN), gonadotropin hormone (GHR). Blocking the synthesis of NO, which leads to a decrease in deposited NO in tissues by 50...70%, results in a change in expression of most of the studied genes. Basically, there was a decrease in gene expression, in particular, myostatin (MSTN), which is responsible for suppressing growth and differentiation of muscle tissue. Thus, nitric oxide in an avian embryo can primarily play the role of regulator of muscle tissue growth, which is important for fast-growing breeds since myoblast proliferation occurs at embryonic stage of development. Regulation can be carried out by activating the mechanisms of oxidation of NO to nitrate which occurs in the embryos of fast-growing breeds.

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Introduction

According to several researchers, nitric oxide (NO) affects the expression of a number of genes [1—3], including genes associated with myogenesis [4]. Until now, it has not been possible to quantitatively evaluate this effect and determine the prospects for its use, since monitoring the content of NO metabolites in tissues is methodologically complicated. Using a highly sensitive and highly specific enzyme sensor that allows such monitoring [5], we found that in avian embryo, about 2% of arginine is spent on the synthesis of nitric oxide [6]. The intensity of NO synthesis is approximately the same in all embryos of birds of the same species. However, in embryos of meat forms, NO is predominantly oxidized to nitrate, while in embryos of egg forms it accumulates in so-called NO donor compounds: S-nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC), high-molecular nitrates (RNO_2). The difference in the ratio of nitrate and donor compounds in the embryo reaches two orders of magnitude [6, 7].

Obviously, nitric oxide is synthesized in excess in bird embryo to ensure vital physiological processes. This is evidenced by the fact that in the embryos of meat forms, NO is mainly oxidized to nitrate [6]. It was shown that some histological indices of embryonic muscles correlate with the degree of NO oxidation in the embryo [6]. Considering data on the effect of NO on gene expression [1—4], it can be assumed that the main part of nitric oxide in embryo acts as a regulator of gene expression, in particular, those responsible for myogenesis. We have the opportunity to quantitatively assess this effect and practically use it in poultry farming.

The purpose of the study was to clarify the relationship between NO content in tissues of bird embryos and expression of genes responsible for myogenesis at various stages of embryogenesis.

Materials and methods

Fertilized eggs of the following chicken breeds and crosses were used in the experiments: cross 'Smena 9' (Cornish breed (line SM 56), Plymouth Rock breed (line

SM 79) and the original hybrid), crosses ‘Hisex White’ and ‘Hisex Brown’, breeds ‘Mini meat’ (line A77), ‘Kulangi’, ‘Brama Palevaya’, ‘Yurlovskaya Golosistaya’ and ‘Orlovskaya’; and quails of ‘Japanese gray’ breed obtained in “Genofond” (Russia). Incubation of eggs was carried out in conditions of the hatchery (“Zagorskoye”, Sergiev Posad, Moscow region). Incubators IPH-10 (Russia) were used. The temperature during the incubation period (from the 1st to the 18th day of incubation) was 37.6 °C, during the hatching period (from the 19th to the 21st day of incubation) — 37.2 °C in accordance with the recommendations of Russian Research and Technological Poultry Institute [10].

In all cases, 5...8 embryos of each breed or cross were selected to determine gene expression.

To determine the content of nitro- and nitroso compounds in homogenates, an enzyme sensor was used, the action of which is based on the unique ability of nitrite and other nitroso compounds containing NO⁺ group to inhibit catalase in presence of halogen ions with approximately equal efficiency, equally dependent on pH of the environment [5–8]. The ability to inhibit the enzyme is lost under the influence of several substances specific to each group of nitroso compounds [5]. To determine nitro compounds, vanadium trichloride was used to reduce the nitro group to a nitroso group, which can inhibit catalase. The sensitivity of the method is 40 nM [5, 7].

Gene expression was studied using the RT-PCR method [9]. The housekeeping gene TATA-binding protein (TBP) was used as a control, since nitroarginine and arginine had no effect on its expression. RNA was isolated from homogenized tissue and synthesized to DNA using the PCR method with reverse transcription. The expression of candidate genes in quantitative RT-PCR detection was calculated relative to the control gene TBP using the $2^{-\Delta\Delta C_T}$ method [9], where C_t is the PCR cycle in which the fluorescent signal from the dye crosses the established threshold level. Thus, the smaller the C_t value, the more intense the amplification process and, consequently, the higher the gene expression.

On the 6th–7th day of embryogenesis, due to the complexity of muscle tissue selection, embryo homogenate was obtained using a glass homogenizer DWK Life Sciences GmbH, Germany (8 min, 40 frictions/min, 6 °C). On the 13th–14th day of development, homogenate of the chest and femoral muscles of the embryo was obtained using an Oster tissue grinder (Mexico).

To establish relationship between the content of deposited NO in tissues and the expression of the studied genes, solutions of exogenous substances were introduced into the eggs before laying and on the 11th day of incubation: a nitric oxide synthesis blocker — nitroarginine (H), as well as a mixture of nitroarginine and a nitric oxide synthesis substrate — arginine (H + A). The dosage is indicated in Tables 3, 4. Sterile saline was introduced into the control group (C) at the same time. The introduction into the egg was carried out through the shell from the side of the air chamber with insulin syringes. N ω -nitro-L-arginine and L-arginine Merck were used, dilution was carried out in sterile physiological saline.

For statistical processing, the BioStat software package was used.

Incubation was carried out in vivarium conditions (“Zagorskoye”, Russian Research and Technological Poultry Institute, Sergiev Posad, Moscow region). IPH-10

incubators (Russia) were used. The temperature during the incubation period (1st to 18th day of incubation) was 37.6 °C, during the hatching period (19th to 21st day of incubation) — 37.2 °C in accordance with the recommendations of Russian Research and Technological Poultry Institute.

For statistical processing, the BioStat software package was used.

Results and discussion

Embryos of ten chicken breeds, lines and crosses characterized by high (80% and more — group 1) and low (2...4% — group 2) degree of NO oxidation in embryonic tissues were studied. On average, the expression value of most genes in embryos of meat and fighting forms, distinguished by a high degree of oxidation of nitric oxide to nitrate in tissues, was lower than in embryos of egg forms, distinguished by insignificant degree of oxidation. According to the expression of myogenic differentiation factor 1 (myoD1), myogenesis factor 5 (myf 5) and myogenin (myog), there were reliable differences between these groups according to the Mann — Whitney criterion (Tables 1, 2).

Table 1

Expression of genes: myocyte proliferation factor 2c (mef 2c), myogenic differentiation 1 (myoD1), myogenesis factor 5 (myf 5), myosin (mhy 1), myogenin (myog), somatostatin (MSTN), gonadotropic hormone (GHR) — in tissue of pectoral muscles of chicken embryos of various breeds, lines and crosses on the 14th day of incubation* (n = 7)

Breed, lines, cross	NO ₃ ⁻ / NO, %	DonorsNO+nitrate, μM	MSTN	GHR	mef2c	myog	mhy 1	myoD1	myf 5
Group 1									
Mini meat (line A77 group 2)	98.7±1.8	495.5±21.1	-1.5±0.7	-1.3±0.5	-1.6±0.5	0.7±0.4	5.4±0.6	-0.5±0.5	10.1±0.5
SM56 'Cornish'	97.6±2.7	519.8±17.6	-2.5±0.4	-2.6±0.3	-1.4±0.3	2.0±0.4	4.2±0.5	-1.2±0.5	9.3±0.4
Kulangi	95.7±2.5	528.5±17.7	-0.2±0.4	-1.2±0.6	-0.6±0.5	7.2±1.3	13.3±1.1	6.4±0.4	2.0±0.2
Smena 9	97.9 ±2.6	572.7±18.5	-3.8±0.4	-2.5±0.3	-2.6±0.3	-2.8±0.3	15.8±1.7	-3.4±0.4	3.1±0.3
Brama palevaya	80.4±2.9	501.1±17.1	-5.2±1.0	-4.9±0.5	-7.9±2.9	1.1±0.5	0.7±0.5	4.6±0.7	2.9±0.5
Group 2									
Hisex White	1.8±1.0	474.6±16.6	-1.3±0.9	-2.3±0.6	-2.0±1.1	-0.3±0.8	4.7±0.4	-2.1±0.6	5.9±0.6
Plymouth Rock SM79	2.9±1.3	461.7±18.2	-2.8±0.5	-2.2±0.7	-2.1±1.3	-6.2±0.8	4.8±0.5	-1.6±1.1	2.4±0.8
Yurlovskaya	3.6±1.9	448.1±15.3	-6.7±0.5	-6.1±1.1	-8.1±0.6	-1.1±0.3	-0.3±0.2	2.9±0.8	-0.3±0.2
Hisex Brown	2.3±0.8	474.6±16.6	0.4±0.4	-2.6±0.4	-2.7±1.2	-0.8±0.7	0.4±0.3	-4.6±0.7	-0.6±0.4
Orlovskaya	1.9±1.1	444.1±14.2	-1.4±0.8	-1.7±0.4	-1.1±0.8	-2.6±0.7	13.3±2.3	-4.1±0.4	5.1±1.0
								u _{exp} < u _{crit}	u _{exp} < u _{crit}

*Table 1 shows ΔCt = Ct — Ct TBP.

Source: compiled by V. Yu. Titov, I.I. Kochish, O.V. Myasnikova, A.M. Dolgorukova, M.A. Zaitseva, P.S. Romanov, H.R. Vardanyan.

Table 2

Expression of genes: myocyte proliferation factor 2c (mef 2c), myogenic differentiation 1 (myoD1), myogenesis factor 5 (myf 5), myosin (mhy 1), myogenin (myog), somatostatin (MSTN), gonadotropic hormone (GHR) – in leg muscle tissue of chicken embryos* ($n = 7$)

Breed, lines, cross	NO ₃ ⁻ / NO, %	DonorsNO+nitrate, μM	MSTN	GHR	mef2c	myog	mhy 1	myoD1	myf 5
Group 1									
Mini meat	97.6±1.8	501.5±18.2	0.4±0.8	0.7±0.2	-2.3±1.8	1.4±1.0	6.8±1.3	0.9±1.2	11.5±1.7
SM56									
Cornish Line 'Smena 9'	95.8±2.9	520.1±16.4	-1.9±0.3	-1.7±0.4	0.7±0.4	12.0±1.7	8.3±2.6	3.4±0.8	13.4±2.1
Kulangi	95.2±2.6	528.5±17.7	-1.8±0.9	-2.2±1.8	2.9±1.7	7.0±1.6	14.3±2.0	3.9±0.6	1.4±0.9
'Smena9'	98.1 ±2.6	561.7±18.7	-2.1±0.4	-1.7±0.5	-1.1±0.8	-2.6±1.4	12.6±2.3	-4.1±1.2	2.8±0.4
Brama	78.5±3.0	495.5±16.6	-3.3±0.5	-3.2±1.4	-7.1±0.9	0.5±0.3	-0.2±0.1	-4.3±0.7	-5.8±0.6
Group 2									
Hisex White	2.4±1.1	442.5±15.8	-0.4±0.2	-0.4±0.1	-1.3±0.5	-0.2±0.1	6.2±0.2	-2.5±0.4	6.7±1.5
Plymouth rock SM79, line 'Smena 9'	3.1±1.8	493.2±17.4	-2.1±0.2	-1.6±0.3	0.0±0.1	-0.4±0.1	6.7±2.2	-3.8±0.5	5.0±0.8
Yurlovskaya	3.8±1.8	434.7±16.1	-5.6±0.6	-4.7±0.6	-9.0±1.4	-0.6±0.2	-1.1±0.8	-4.7±0.4	-7.5±1.4
Hisex Brown	2.3±1.3	451.6±17.2	-1.0±0.9	-1.2±2.2	0.8±0.2	0.4±1.0	10.1±2.4	-0.4±1.8	2.9±2.3
Orlovskaya	2.3±1.2	428.3±13.1	0.4±0.2	-0.7±0.3	-0.8±0.2	-1.8±0.3	12.4±1.9	-2.5±0.4	4.3±0.8
						$u_{exp} < u_{crit}$			

* Table 2 shows $\Delta Ct = Ct - Ct \text{ TBP}$.

Source: compiled by V. Yu. Titov, I.I. Kochish, O.V. Myasnikova, A.M. Dolgorukova, M.A. Zaitseva, P.S. Romanov, H.R. Vardanyan.

Within these groups, large differences in gene expression were found. For example, myostatin expression varied from -1.2 to -6.9 in group 2 and from -1.6 to -5.4 in group 1 in pectoral muscles (Table 1). The differences in leg muscles were no smaller (Table 2). There were significant differences between these groups according to the Mann-Whitney criterion in expression of myogenic differentiation factor 1 (myoD1) and myogenesis factor 5 (myf 5) in pectoral muscles and myogenin (myog) in leg muscles.

In this regard, to establish relationship between the content of deposited NO in tissues and the expression of the genes studied, the most informative seems to be varying the concentration of deposited NO within one breed, line and cross, using blockers of NO synthesis and the substrate of its synthesis — arginine.

Nitroarginine effectively inhibits NO synthesis, which leads to decrease in content of NO metabolites in embryonic tissues, without changing the degree of oxidation of synthesized NO to nitrate (Tables 3, 4), which is determined by peculiarities of embryonic tissues [6]. But the effect of H is not constant. Introduced before incubation, it effectively inhibits NO synthesis in chicken and quail embryos for up to 11 days. Then its effect disappears but resumes after repeated introduction into the egg [6]. H is a competitive inhibitor competing for NO synthase with arginine (A),

a substrate for NO synthesis. Introduction of A into the egg together with H in a concentration 10 times greater than H completely removed the inhibitory effect of the latter (Tables 3, 4).

As we have established, A in the egg is at a saturation concentration for NO synthase [6]. Its introduction into the egg does not lead to intensification of NO synthesis, but removes the effect of H (Tables 3, 4).

H introduced before incubation provided decrease in intensity of NO metabolite accumulation in embryo by 7 days by approximately 70% (Tables 3, 4). At the same time, in the tissues of chicken embryos, as well as quails, there was a decrease in expression of most of the studied genes, which did not change if arginine (H + A) was introduced into the egg together with H. The content of NO metabolites in homogenates also does not change when H + A is introduced. A, introduced into the egg without H, did not have a reliable effect either on gene expression (Table 3) or on content of NO metabolites in embryo tissues (Tables 3, 4).

Table 3

The effect of NO synthesis blocker nitroarginine on expression of some genes in 7- and 14-day embryos of chickens of X2 line of Hisex White cross*

Group	NO ₃ ⁻ / NO, %	DonorsNO+ nitrate, μM	Expression (gene-TBP)						
			MSTN	Myf5	Mef2	GHR	Myog	MyoD1	Mhy1
7 days									
K	1.5 ± 0.8	162.2±8.4	-2.45±0.25	-1.12±0.15	-0.21±0.23	-1.85±0.21	0.38±0.07	-1.15±0.18	-2.61±0.24
H	1.4±1.1	54.8±5.5 <i>p</i> <0.05	-1.38±0.07 <i>p</i> <0.05	-0.07±0.04	0.48±0.13 <i>p</i> <0.05	-1.08±0.26 <i>p</i> <0.05	0.23±0.06	-0.25±0.06 <i>p</i> <0.05	-0.58±0.14 <i>p</i> <0.05
H+A	1.6±0.9	167.1±8.8	-2.53±0.1	-0.77±0.11	-0.91±0.22	-2.01±0.28	-0.13±0.09	-0.70±0.13	-2.08±0.14
A	1.6±1.0	171.1±9.9	-2.49±0.15	-0.87±0.13	-0.58±0.22	-1.96±0.27	-0.08±0.07	-0.96±0.16	-2.91±0.26
14 days, pectoral muscles									
K	2.5±1.2	465.7±10.3	-2.43±0.38	1.31±0.05	-0.03±0.22	-1.85±0.34	-1.02±0.19	-1.83±0.22	-3.72±0.15
H	2.6±1.2	250.2±9.5 <i>p</i> <0.05	-1.18±0.06 <i>p</i> <0.05	1.67±0.29	0.53±0.17 <i>p</i> <0.05	-1.01±0.18 <i>p</i> <0.05	-0.54±0.16 <i>p</i> <0.05	-0.80±0.20 <i>p</i> <0.05	-2.93±0.12 <i>p</i> <0.05
H+A	2.8±1.5	462.4±11.2	-1.56±0.09	-1.12±0.06	-0.13±0.23	-1.35±0.09	-2.30±0.31	-1.91±0.27	-3.52±0.20
14 days, leg muscles									
K	2.5±1.2	475.7±10.3	-1.36±0.08	-0.42±0.14	1.37±0.24	0.12±0.1	-1.60±0.26	-0.44±0.27	-2.47±0.44
H	2.6±1.2	239.2±8.5 <i>p</i> <0.05	-0.14±0.22 <i>p</i> <0.05	1.21±0.21 <i>p</i> <0.05	1.42±0.33	0.57±0.35	-1.05±0.14 <i>p</i> <0.05	0.01±0.15 <i>p</i> <0.05	-0.35±0.19 <i>p</i> <0.05
H+A	2.8±1.5	452.3±11.2	-1.07±0.18	-0.51±0.30	0.1±0.19	-1.08±0.14	-2.29±0.27	-0.76±0.26	-1.64±0.41

*0.3 ml of 30 mM nitroarginine was added to the eggs of group H before incubation and on the 11th day; 0.15 ml of 60 mM nitroarginine + 0.15 ml of 600 mM arginine were added to the eggs of group H+A before incubation and on the 11th day; 0.3 ml of sterile saline was added to the control (C) eggs at the same time. 0.3 ml of 300 mM arginine was added to group A before incubation.

Source: compiled by V. Yu. Titov, I.I. Kochish, O.V. Myasnikova, A.M. Dolgorukova, M.A. Zaitseva, P.S. Romanov, H.R. Vardanyan.

Of the genes whose expression was significantly affected by H, we note first myostatin (MSTN). In chickens, it decreased more than 2-fold on the 7th day (Table3), and in

quails, it decreased 7-fold on the 6th day (Table 4). Also, expression of gonadotropic hormone (GHR) decreased 1.3-fold in quails and 2-fold in chickens. The effect on expression of other genes is not always unambiguous. Thus, there was no significant effect on the expression of myogenesis factor 5 (myf5) in quails (Table 4), and on the verge of significance — in chickens (Table 3). There was a significant effect on expression of myocyte proliferation factor 2 (mef2) in chickens — a decrease of 1.6–1.7 times. There was no significant effect in quails (Table 4). There was no significant effect on myogenin (myog) expression in chickens on the 7th day. In quails, there was a significant decrease in expression by 1.5 times. No significant effect on expression of myogenic differentiation factor 1 (myoD1) was observed in chickens on the 7th day. Myosin (mhy 1) expression in chickens significantly decreased by 2–4 times. This gene is not present in quails (Tables 3, 4).

H, administered repeatedly on the 11th day, caused a decrease in accumulation of NO metabolites in embryonic tissues by 50...60% on the 14th day (Tables 3, 4). This was accompanied by change in expression of the studied genes, primarily myostatin (MSTN) and gonadotropic hormone (GHR). In hens, MSTN expression under the influence of H decreased by 2.3–2.4 times in tissues of pectoral and leg muscles (Table 3), in quails in pectoral muscles — by 1.17 times, in leg muscles — by 1.9 (Table 4). GHR expression decreased in hens by 1.4...1.7 times, in quails in pectoral muscles the expression decreased by 1.5 times, in leg muscles — by 2.7 times (Tables 3, 4). The effects on other genes were also preserved. Note that the differences in gene expression between the pectoral and leg muscles are significant in some cases, even though there is no reliable difference in the content of NO metabolites between them.

Table 4

Effect of NO synthesis blocker nitroarginine on expression of some genes in 6- and 13-day-old embryos of Japanese gray quail*

Group	NO ₃ ⁻ / NO, %	DonorsNO+ nitrate, μM	Expression (gene–TBP)					
			MSTN	Myf5	Mef2	GHR	Myog	MyoD1
6 days								
K	1.1 ± 0.8	149.3±7.9	−0.61±0.06	2.82±0.06	−1.56±0.08	−0.20±0.08	−0.80±0.30	0.05±0.05
H	1.2±0.8	28.1±2.1 <i>p</i> <0.05	2.20±0.80 <i>p</i> <0.05	2.94±0.10	−1.22±0.09	0.21±0.10 <i>p</i> <0.05	−0.20±0.05 <i>p</i> <0.05	0.15±0.10
H+A	1.1±0.8	150.5±8.1	−1.48±0.18	2.46±0.08	−1.10±0.11	−0.15±0.18	−0.74±0.09	0.21±0.07
13 days, pectoral muscles								
K	2.5±1.5	576.6±13.3	0.20±0.16	2.29±0.13	0.21±0.07	0.91±0.12	0.89±0.18	0.66±0.12
H	2.4±1.2	322.4±11.2 <i>p</i> <0.05	0.42±0.08	2.39±0.13	0.18±0.07	1.54±0.04 <i>p</i> <0.05	1.21±0.13	0.91±0.17
13 days, leg muscles								
K	2.4±1.5	565.4±12.8	−0.43±0.13	2.20±0.13	−0.27±0.07	0.15±0.18	0.21±0.10	0.38±0.06
H	2.3±1.4	308.1±12.8 <i>p</i> <0.05	0.48±0.20 <i>p</i> <0.05	2.71±0.13	0.39±0.17 <i>p</i> <0.05	1.60±0.24 <i>p</i> <0.05	1.24±0.14 <i>p</i> <0.05	1.14±0.09 <i>p</i> <0.05

*0.15 ml of 15 mM nitroarginine was added to the eggs of group H before incubation and on the 11th day. In group H+A, 0.15 ml (30 mM nitroarginine + 300 mM arginine 1:1) was added before incubation and on the 11th day. In the control group (C), 0.3 ml of sterile saline was added at the same time.

Source: compiled by V. Yu. Titov, I.I. Kochish, O.V. Myasnikova, A.M. Dolgorukova, M.A. Zaitseva, P.S. Romanov, H.R. Vardanyan.

The introduction of H + A removed these effects, as well as the effect of H on reducing the concentration of NO metabolites (see Tables 3, 4).

There is a point of view in the literature that NO has an epigenetic effect, influencing the methylation of histone proteins, inhibiting enzymes that carry out demethylation [2]. There is a reason to believe that in bird embryos, the mechanism of NO oxidation to nitrate acts as an expression regulator. Based on the analysis of inheritance of degree of NO oxidation in the embryo, this process is determined by at least two genes [6]. That is, the genes that determine activation of NO oxidation are inherited.

The only thing that can be said about the mechanism of this oxidation is that nitrite does not accumulate in tissues [6]. Consequently, oxidation occurs without generating active forms of nitrogen into the environment. To date, we do not know a way to artificially reduce the intensity of this oxidation. But there is a way to artificially increase it. This is irradiation of incubated embryos with green light, which leads to some increase in the growth rate [10—12], as well as to a reliable increase in the oxidation state of embryonic NO [6, 7]. The effects on gene expression that we observed under irradiation were similar for some genes to those observed under the action of NO synthase blocker [7]. We do not risk considering irradiation with green light as a complete control, since light can have other effects not associated with NO. However, a comparison of this effect with the effect of nitroarginine, which blocks NO synthesis but does not affect the degree of its oxidation (Tables 3, 4), as well as with the average expression values in egg and meat forms (Tables 1, 2) gives reason to assume that the effect is associated with the concentration of NO donor compounds in tissues. The latter include nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC), and some high-molecular nitrates [5, 13].

Conclusion

Thus, a decrease in content of NO donor compounds leads to a decrease in expression of most of the studied genes responsible for myogenesis. We note decrease in expression of myostatin, responsible for suppression of growth and differentiation of muscle tissue. Consequently, nitric oxide in the bird embryo can play the role of regulator of muscle tissue growth, which is important for fast-growing forms, since myoblast proliferation occurs at the embryonic stage of development. Regulation can be carried out by activating the mechanisms of NO oxidation to nitrate.

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Эффекты оксида азота, связанные с экспрессией ряда генов на эмбриональной стадии развития птиц

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Аннотация. Данные, полученные различными исследователями, свидетельствуют о влиянии оксида азота на экспрессию ряда генов, в частности генов, связанных с миогенезом у птиц. Количественная оценка этого эффекта и изучение перспектив его использования ранее не проводились, поскольку контроль содержания метаболитов NO в тканях представлял методическую сложность. Цель исследования — выявить взаимосвязь содержания NO в тканях птичьих эмбрионов и экспрессии генов, ответственных за миогенез, на различных стадиях эмбриогенеза. Использовался высокочувствительный и высокоспецифичный ферментный сенсор, позволяющий оперативно определять содержание основных метаболитов NO в тканях. Экспрессию генов определяли методом ПЦР-РВ. На эмбрионах кур и перепелов исследовали взаимосвязь содержания NO в тканях и экспрессии 7 генов, участвующих в процессе миогенеза: фактора пролиферации миоцитов 2с (mef 2с), миогенной дифференциации 1 (myoD1), фактора миогенеза 5 (myf 5), миозина (mhy 1), миогенина (myog), соматостатина (MSTN), гонадотропного гормона (GHR). Установлено, что блокирование синтеза NO, снижающее содержание депонированного NO в тканях на 50...70%, приводит к изменению экспрессии большинства исследованных генов в несколько раз.

В основном имело место снижение экспрессии генов, в частности, миостатина (MSTN), ответственного за подавление роста и дифференциации мышечной ткани. Таким образом, оксид азота в птичьем эмбрионе может играть роль прежде всего регулятора роста мышечной ткани, что важно для быстрорастущих форм поскольку пролиферация миобластов происходит на эмбриональной стадии развития. Регуляция может осуществляться путем активации механизмов окисления NO до нитрата, что имеет место в эмбрионах быстрорастущих форм.

Ключевые слова: птичьи эмбрионы, соединения-доноры NO, эмбриогенез птиц, миогенез

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