Assessing the β_1:β_2 ratio of sensitized cardiac β adrenergic receptors in the embryonic cardiac tissue of hypoxia-treated Broiler chicken (*Gallus gallus domesticus)*

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Background

A developing embryo is sensitive to alterations and perturbation in the environment, such as nutritional insufficiencies and environmental inadequacies, that could potentially cause significant ramifications in the future growth and survivability of the organism as an adult. Prenatal stress is an important etiological factor in the onset of adult hypertension but the mechanisms responsible for this are poorly understood.

β adrenergic receptors (βAR) modulate the inotropic and chronotropic activity of the heart. Catecholamines mediate the regulation of cardiac performance through the positive stimulation of these receptors. Under periods of stress, such as exposure to hypoxic conditions during embryonic growth, chronic elevated levels of circulating plasma catecholamines hyperstimulate the βARs, triggering physiological changes in the heart. Mammalian and avian studies demonstrate that chronic prenatal hypoxia sensitizes βARs in the embryonic heart but causes postnatal desensitization[1,2,3].

A potential explanation for these differences in sensitivity is a shift in the relative expression of β_1AR and β_2ARs, measured with respect to the relative gene expression of these receptors in the cardiomyocytes of the developing embryos at different developmental ages.

Materials and Methods

The study was conducted on fertilized eggs incubated under two experimental conditions: incubation in normoxia (21 % O_2) and in chronic isobaric hypoxia (14 % O_2). Embryos were sampled at two developmental ages: 15 days and 19 days. Giving four experimental groups (n=8 in each): 15 days Normoxic (15N), 15 days Hypoxic (15H), 19 days Normoxic (19N) and 19 days Hypoxic (19H). qPCR analysis of the β_1AR, β_2AR and housekeeper gene, GAPDH mRNA expression levels of the 15 day and 19 day embryonic chicken cardiac tissue samples was performed. The difference in mRNA expression of each gene was evaluated.

Results

qPCR expression analysis

Treatment	*β_1 (copies)*	*t-test (P)*	*β_2 (copies)*	*t-test (P)*	*GAPDH (copies)*	*t-test (P)*	*β_1/GAPDH ($r\beta_1$)*	*t-test (P)*	*β_2/GAPDH ($r\beta_2$)*	*t-test (P)*	*$r\beta_1/r\beta_2$*	*t-test (P)*
15N	0.33 ± 0.15		0.15 ± 0.08		0.18 ± 0.08		1.79 ± 0.46		0.94 ± 0.41		0.68 ± 0.73	
15H	3.03 ± 1.00	0.0001	1.33 ± 0.60	0.0004	2.19 ± 0.97	0.0003	1.59 ± 0.88	ns	0.63 ± 0.26	0.0490	2.58 ± 0.87	ns
19N	7.09 ± 1.93		4.67 ± 1.97		3.04 ± 1.12		2.69 ± 1.33		1.58 ± 0.71		3.82 ± 1.31	
19H	8.20 ± 3.37	ns	6.24 ± 2.86	ns	4.57 ± 1.26	ns	1.94 ± 1.11	ns	1.40 ± 0.60	ns	1.48 ± 0.58	ns

Table 1. Results of the qPCR analysis for β_1, β_2, and GAPDH, and the calculated relative mRNA expression levels of these genes in chicken fetal cardiomyocytes. Data as mean ± standard error. Significance indicated at $P < 0.05$. 'ns' denotes no significance found between control and treated samples.

Effects of hypoxia on relative β_1:β_2 expression ($r\beta_1/r\beta_2$)

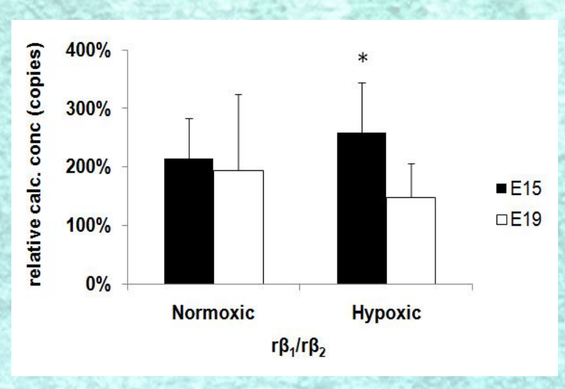

Figure 1. Relative expression of β_1:β_2 adrenergic receptors ($r\beta_1/r\beta_2$) of embryonic chicken cardiomyocytes exposed to normoxic or hypoxic conditions. The relative calculated concentration expressed in percent of the concentration of β_1 expressed relative to β_2. Developmental age is expressed as days of embryonic development. $P < 0.05$, significance between relative β_1:β_2 concentration of the samples between developmental age groups, same treatment (*).

Conclusions

Effects of hypoxia on βAR expression: qPCR expression analysis reveals a significant increase in the number of βARs for both β_1AR and β_2ARs in the 15 day embryos, where there is a 10-fold difference in their expression in the hypoxic-treated relative to the normoxic-treated samples. This vast increase in βARs in the hypoxic samples is not seen in the 19 day embryos.

Effects of age on hypoxic βAR expression: When comparing age there is a significant difference in the normalized relative β_1:β_2 AR ratio seen in the hypoxic samples. The relative β_1:β_2 expression increases in 15H but decreases in 19H, compared to the normoxic samples for each age.

From this we conclude that chronic hypoxia alters the βAR subtype ratio in the heart, possibly explaining the changes in βARs sensitivity pre- and postnatally in response to catecholaminergic hyperstimulation.

References

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