BIOPHYSICS AND BIOCHEMISTRY

Differences in Hormonal Status between Rats with High and Low Resistance to Hypoxia

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Different animals of the same species, including rats, may differ in their resistance to acute hypobaric hypoxia [2]. At present, searches for mechanisms by which the natural resistance of the body to hypoxia is established and maintained are being intensively pursued at the cellular level with a view to the subsequent utilization of identified mechanisms for repairing hypoxic or ischemic damage. It is possible, however, that individual differences in sensitivity to hypoxia are mainly due to varying reactions occurring at the level of regulatory systems, the hormonal system in particular. Also, there appear to be sex-related differences in resistance to hypoxia; some female rats, for example, have been found to be much more resistant than males [6].

In the work reported here we made an attempt to detect possible differences in hormonal status between animals with high and low resistance to hypoxia by measuring blood levels of steroid hormones. These hormones were selected for study because, on the one hand, they are likely to be instrumental in establishing natural resistance to hypoxia and, on the other, glucocorticoids are possibly implicated in the pathogenesis of brain ischemia and hypoxia [5].

MATERIALS AND METHODS

The experiments were conducted during the spring and summer months on two groups of randombred male rats (200-220 g in weight), 42 animals in each, with high and low resistance to acute hypobaric hypoxia (HR and LR groups, respectively), as established by the method described in detail elsewhere [2]. Briefly, the rats were placed in a pressure chamber and briefly (for 1 min) raised to an "altitude" of 11,000 m. At this altitude, excitation (in the form of anxiety), forced respiration, assumption of a lateral position, convulsions, atony of the hind limbs, and respiratory arrest were sequentially observed in the rats. The criterion by which resistance to hypoxia was judged was the time (T) elapsing between the start of exposure to it and the second apneic inspiration. The HR group comprised rats with a T of more than 9 min and the LR group, those with a T of less than 3 min.

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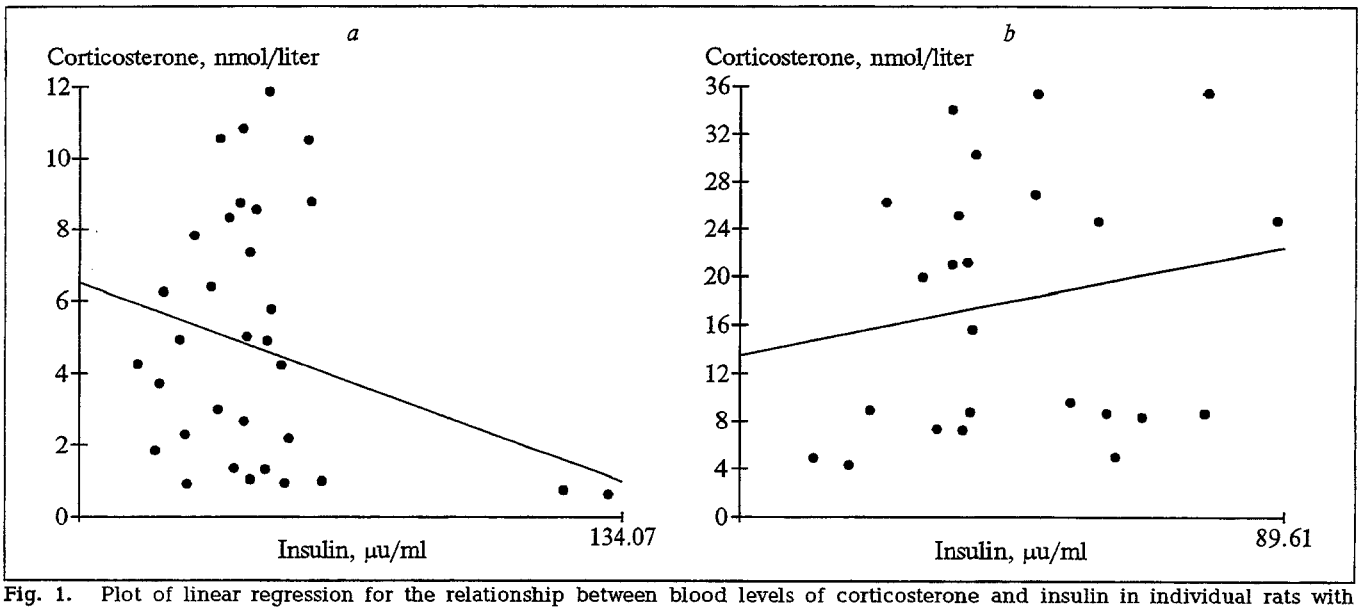

Fig. 1. Plot of linear regression for the relationship between blood levels of corticosterone and insulin in individual rats with high (*a*) and low (*b*) resistance to hypoxia: pooled data for morning (09:00 – 12:00) and early afternoon (13:00 – 14:00) hours.

After a 2-week rehabilitation period, the rats exposed to acute hypoxia as described above were killed by decapitation at different times of the day (09:00-12:00 h, 13:00-14:00 h, and 15:00-17:00 h). Blood from all rats was collected into plastic test tubes precooled on ice and containing a 6% EDTA-Na solution (0.1 ml per 5 ml blood). The test tubes were immediately centrifuged at 3000 g for 15 min at 4°C, and the plasma samples obtained were stored at -20°C until assayed for hormones (corticosterone, insulin, and testosterone) by standard radioimmunological techniques using kits purchased from Russian manufacturers.

RESULTS

Although, in general, no statistically significant differences in blood levels of corticosterone, insulin, or testosterone were detected between the LR and HR groups, the LR group was found to show subtle differences from the HR group in hormonal status at different times of the day. As follows from Table 1, at 15:00-17:00 h the corticosterone levels in LR rats were 72% higher, on average, than in HR rats; in LR rats, unlike in HR rats, the blood levels of this hormone at 15:00-17:00 h were 2.1 to 2.4 times higher than earlier in the day. In contrast, the two groups did not differ significantly in insulin levels, although within each group the levels of this hormone at 15.00-17.00 h differed significantly from those at 09:00-12:00 h and 13:00-14:00 h. No significant differences, either between or within the HR and LR groups, were detected in testosterone, yet its circadian rhythm was not lost.

Thus, in our study the circadian rhythm of corticosterone, described in the literature for this hormone, was more strongly marked in LR than

TABLE 1. Blood Levels of Corticosterone, Insulin, and Testosterone at Various Times of the Day in Rats with High (HR) and Low (LR) Resistance to Hypoxia (*M*±*m*).

HR rats (n=42)			LR rats (n=42)		
9:00-12:00 h	13:00-14:00 h	15:00-17:00 h	9:00-12:00 h	13:00-14:00 h	15:00-17:00 h
Corticosterone (nmol/liter)					
102.3±7.8 (n=24)	117.3±21.7 (n=6)	123.3±26.3 (n=6)	86.7±2.3 (n=18)	99.5±32.8 (n=6)	211.8±20.6* (n=18)
Insulin (μU/ml)					
35.7±2.5 (n=24)	36.6±4.6 (n=6)	58.7±8.6** (n=6)	39.9±4.3 (n=18)	42.4±9.2 (n=6)	60.2±11.8** (n=18)
Testosterone (ng/ml)					
1.19±0.12 (n=27)	0.23±0.01 (n=6)	0.92±0.23 (n=6)	1.20±0.19 (n=14)	0.47±0.17 (n=6)	0.98±0.20 (n=18)

Note. *p<0.05 in comparison with HR rats; **p<0.05 in comparison with insulin levels at 9 – 12 h and 13 – 14 h in rats of the same (HR or LR) group.

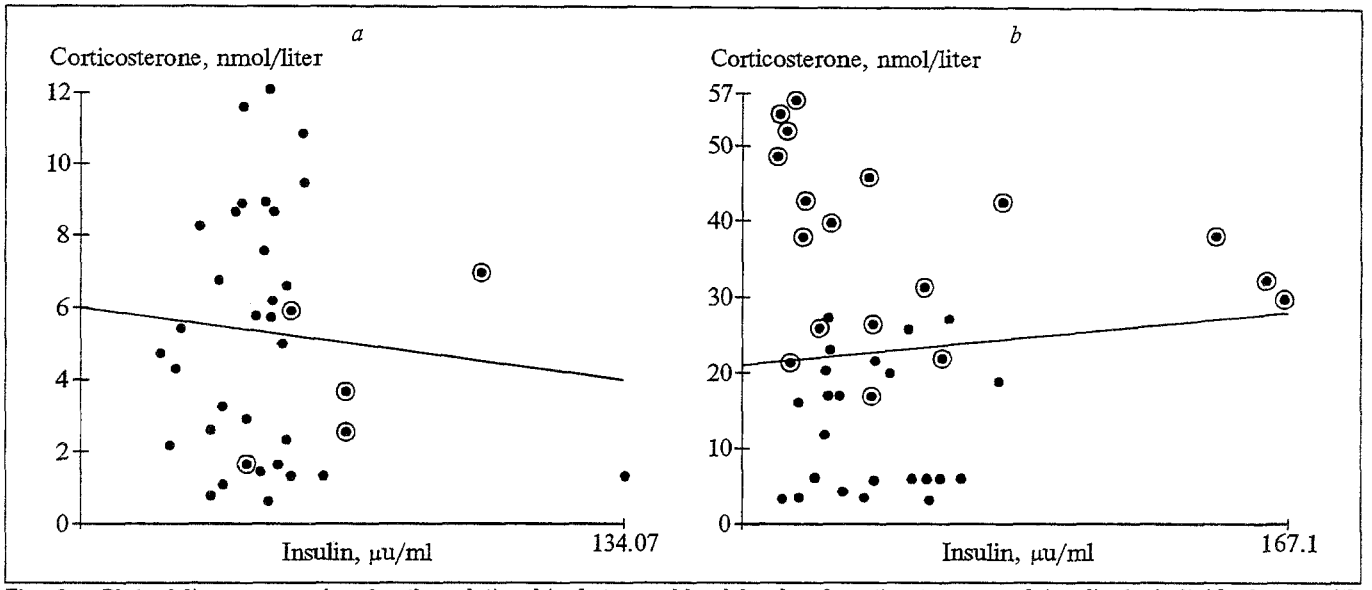

Fig. 2. Plot of linear regression for the relationship between blood levels of corticosterone and insulin in individual rats with high and low resistance to hypoxia: pooled data for morning (09:00 – 12:00), early afternoon (13:00 – 14:00), and late afternoon (15:00 – 17:00) hours. Values for 15:00 – 17:00 are encircled.

in HR rats, which appears to reflect the greater "strain" of the hypothalamus-pituitary-adrenal axis in the LR group of animals during the active period of the day (15:00-17:00 h).

Significant differences in corticosterone levels between LR and HR rats occurred during the morning hours (between 09:00 and 12:00) in tests where rats exposed to acute hypobaric hypoxia as described above were additionally exposed to such hypoxia for 1.5 min (Table 2). This indicates that the blood level of corticosterone in LR rats is more labile than in HR animals, and that the stress reaction in LR rats to the 1.5-minute hypoxia involved the hormonal component to a greater extent than in their HR counterparts.

The integrated physiological actions of glucocorticoids are known to result in relative hyperglycemia, negative nitrogen balance, and fatty tissue atrophy - effects which are directly opposite to those of insulin [4]. It has also been demonstrated that insulin and glucocorticoids produce opposite effects on amino-acid transport and protein degradation [7]. Moreover, glucocorticoids antagonize the antilipolytic action of insulin by preventing its binding to fatty tissue cells. By altering the blood level of glucose, glucocorticoids alter insulin release and, in addition, can inhibit insulin secretion in a more direct way by acting on the α-adrenergic receptors of β-cells in the islets of Langerhans [3]. All this indicates that the physiological actions of glucocorticoids on metabolic processes are antagonistic to those of insulin; hence, a negative correlation between their blood levels may be expected [1].

TABLE 2. Effect of 1.5 – Minute Hypoxia on Blood Corticosterone Levels (nmol/liter) in High – Resistance (HR) and Low – Resistance (LR) Rats. The Values are Means ± SEM

HR rats		LR rats	
intact	hypoxic	intact	hypoxic
102.3±7.5 (n=24)	137.2±11.6 (n=6)	86.7±2.3 (n=18)	163.9±43.2*,** (n=23)

Note. Intact rats were those which had been rehabilitated after being tested for resistance to hypoxia (as outlined above) and then killed in the morning hours (between 09:00 and 12:00). The hypoxic rats were those exposed additionally to acute hypoxia for 1.5 min by being raised to an "altitude of 11,000 m in a pressure chamber and then killed at 09:00 – 12:00 h. *p<0.05 in comparison with HR rats; **p<0.05 in comparison with intact LR rats.

It is therefore essential to take into consideration individual characteristics of the animals in studies designed to evaluate the involvement of hormones in the mechanisms of natural resistance to hypoxia. In this respect, it may be useful to examine the relationship between blood levels of corticosterone and insulin - two hormones with important roles in the regulation of energy balance in the cells. As shown in Figs. 1 and 2, there is a high positive coefficient of correlation (r) between the blood levels of corticosterone and insulin for individual LR rats and a negative r for HR rats, which indicates that these rats differed substantially in the profile of hormones that participate in the hypoxia-induced stress reaction as agents redistributing energy resources in the body.

The main conclusion to be drawn from this study is that hormonal regulation in animals highly resistant to hypoxia differs from that in animals with low resistance. The detected differ-

ences concern hormones involved in the regulation of stress reactions and in the redistribution of energy resources in stressed and hypoxic animals. The LR and HR groups did not differ significantly in blood levels of testosterone.

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Regulation of RNA Polymerase Activity in Liver and Brain Cell Nuclei by a Cytoplasmic Thyroxine Modulator in Rats of Various Ages

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Hormones play important roles in the regulation of the morphological and functional differentiation which cells undergo during ontogeny. The role of the thyroid hormones in this differentiation consists essentially in the activation of protein synthesis in cell nuclei and ribosomes via the nuclear hormone-receptor complex [4,12]. However, regulation of metabolic processes by the thyroid also occurs at the level of numerous membrane structures of the cell, as is indicated by the presence of highly specific binding sites for thyroid hormones in mitochondria [10], cytosol [6], and on plasma membranes [8]. Most of the published information on receptor structures of thyroid hormones has been obtained in studies of mature organisms. The number of investigations examining thyroid hormone receptors in the course of ontogeny is small. The best studied are nuclear receptors of developing tissues [5], and there is some scant information about receptors localized in the cytosol and other cell organelles [1].

Previously, we identified a thyroxine-binding protein designated thyroxine modulator, or T_4M, that mediates certain effects of thyroxine in the nucleus and mitochondria [2,3]. The present study was undertaken to compare the effects of this modulator on RNA polymerase activity in liver and brain cell nuclei of rats during ontogeny.

MATERIALS AND METHODS

For the experiments, 20-day hyperthyroid embryos and 7-, 20-, 45-, and 90-day-old rats of the

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