Modification of Normal Activities of Angiotensin II and Angiotensin IV in Rats with Experimental Hypo- and Hyperglycemia

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Changes in blood glucose levels are paralleled by modification of normal activities of angiotensin II and angiotensin IV. Hypo- and hyperglycemia similarly reduced the hypertensive effect of angiotensin II and similarly distorted the initial hypotensive effect of angiotensin IV. Presumably, the adaptation and compensatory processes in the renin-angiotensin system under conditions of shifted homeostatic constants manifest by phenomena of external reintegration and redistribution of functions of its individual peptide components. This provides restructuring of the mechanisms of intra- and intersystemic organization of physiological functions under extreme conditions.

Key Words: *hemodynamics; angiotensin II; angiotensin IV; acute hypoglycemia; hyperglycemia*

The renin-angiotensin system (RAS) plays an important role in the regulation of the adaptation and compensatory processes, for example, in the maintenance of water-salt balance, hemodynamic regulation, modulation of humoral and cellular immunity, *etc.* [1,5,9,15]. The main effector peptide of RAS, angiotensin II (ATII), is characterized by manifest hypertensive and dipsogenic activities. Another peptide component of RAS, angiotensin IV (ATIV), is a product of enzymatic cleavage of ATII and is characterized by physiological functions of its own: it is involved in modulation of training and memory processes and has neurotrophic effects. The physiological effects of both peptides are mediated through specific AT_1 and AT_2 receptors for ATII and AT_4 receptors for ATIV [2,9,14,15].

Hyperactivation of RAS is associated with the development of many "conformation' diseases, running a protracted course (atherosclerosis, essential hypertension, cardiac and cerebral ischemia, diabetes, *etc.*). The discirculatory disorders associated with these conditions are always closely connected to metabolic shifts, for example, carbohydrate metabolism imbalance [1,2,6,9,10,15]. Glucose and insulin levels are important parameters of carbohydrate metabolism. Many endogenous bioactive substances, including RAS peptide components, are involved in their regulation [2,4,9,10]. That is why it is interesting to investigate physiological activities of ATII and ATIV under conditions of experimental acute disorders of carbohydrate metabolism by inducing hypo- and hyperglycemia in animals.

MATERIALS AND METHODS

Physiological activities of ATII and ATIV under conditions of hypo- and hyperglycemia were analyzed by their effects on hemodynamic parameters.

Experiments were carried out on 58 male Wistar rats (350-400 g). In experimental series I, the animals with acute hypoglycemia (n=16) were intraperitoneally injected with ATII and ATIV (Americanpeptides)

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in doses of 300 and 400 μg/kg, respectively; in experimental series II, the animals with acute hyperglycemia (n=14) received the injections. Acute hypoglycemia was induced by intraperitoneal injection of 1 U insulin (Novo Nordisk) in 1 ml 0.9% NaCl. Acute hyperglycemia was induced by intraperitoneal injections of 2 ml of 40% glucose. Angiotensins were injected 30 min after insulin or glucose injections. Controls (n=28) were injected with saline according to the same protocol. All animals were allowed a free access to water and food.

Hemodynamic parameters, systolic BP (SBP) and HR, were recorded by the indirect method using an NIBP system (AD Instruments) in conscious rats placed into plastic boxes. Because of variability of the initial SBP and HR values in different animals, changes in these parameters in response to exposure were expressed in percent of the basal level. The values of SBP and HR were interpolated with cubic splines for adjusting the data to univariate time series.

The time course of blood glucose was studied in a special series of experiments (n=39) 30, 60, and 90 min after ATII and ATIV injections following insulin or glucose. Blood glucose was measured using test strips and Escensia Entrust glucometer.

All experiments were carried out in accordance with the Helsinki Declaration on Humane Treatment of Animals.

The results were statistically processed using Student's t test. The differences were considered significant at p<0.05.

RESULTS

Blood glucose level in animals before injections of the studied substances was 4.8-5.9 mmol/liter (Tables 1, 2).

Thirty minutes after injection of 1 U insulin, the blood glucose level in rats dropped to 3.3 mmol/liter, by 40% below the basal level (p<0.001). This level of hypoglycemia persisted for 90 min. The time course of blood glucose level after injections of ATII and ATIV after insulin was similar (Table 1).

Administration of 2 ml 40% glucose led to increase of glycemia to 13.2 mmol/liter by min 30, which surpassed the initial level by 200% (p<0.001). The level of glycemia remained high after 60 and 90 min and decreased after 120 min. ATII and ATIV had virtually no effect on the course of hyperglycemia in response to glucose (Table 2).

Studies of the hemodynamics showed that shifts in blood glucose level led to clear-cut changes in the regulation of circulation. Hypertensive activity of ATII decreased significantly (p<0.05) under conditions of hypo- and hyperglycemia: SBP increased by 47% in response to ATII in the controls, while after insulin injection SBP increased by no more than 25% and after glucose by 22% (Fig. 1, *a*). Lasting (1.5 h) tachyarrhythmia observed in control rats under the effect of ATII was virtually completely suppressed in hypoglycemia and even transformed into moderate bradycardia under conditions of acute hyperglycemia (Fig. 1, *b*).

TABLE 1. Blood Glucose Levels (mmol/liter) in Hypoglycemic Rats in Response to ATII and ATIV

Group	Basal level	Time after insulin injection, min			
		30	60	90	120
Insulin+saline	4.80±0.22	3.00±0.38***	3.10±0.50***	2.80±0.61***	4.20±0.43**
Insulin+ATII	5.50±0.39	3.20±0.33***	2.80±0.44***	3.20±0.56***	4.50±0.87*
Insulin+ATIV	5.60±0.38	3.60±0.99**	3.00±0.34***	3.10±0.40***	4.60±0.58*

Note. Here and in Table 2: p<0.05, **p<0.01, ***p<0.001 in comparison with baselilne.

TABLE 2. Blood Glucose Levels in Hyperglycemic Rats (mmol/liter) in Response to ATII and ATIV

Group	Basal level	Time after insulin injection, min			
		30	60	90	120
Glucose+saline	5.40±0.35	13.70±2.51***	9.90±1.64***	7.50±0.81**	6.20±0.58
Glucose+ATII	5.30±0.35	13.00±2.36***	10.20±1.64***	8.20±0.79***	6.20±0.69*
Glucose+ATIV	5.90±0.27	12.80±1.65***	8.10±0.81***	6.10±0.58	6.00±0.48

Our results indicated that in contrast to ATII, ATIV in the studied doses caused a minor hypotensive effect in control animals (a 4-5% reduction of SBP) and had no effect on HR. Acute hypo- and hyperglycemia significantly modified ($p<0.05$) normal activity of ATIV. Moderate hypertension was observed in both experimental series: SBP surpassed the initial values by 13-14% (Fig. 2, *a*). Changes in HR in response to ATIV injected after insulin were biphasic: a slight acceleration of the heart rhythm was followed by bradycardia. By contrast, combined administration of 40% glucose and ATIV caused lasting tachycardia of up to 8% (Fig. 2, *b*).

Hence, the results of our experiments showed that shifts in a most important metabolic constant, blood glucose level, modified normal activity of the studied RAS components. The changes in the activity of each of the peptides did not depend on the direction of shifts in blood glucose levels: hypo- and hyperglycemia similarly reduced the hypertensive effect of ATII and similarly distorted the initially hypotensive effect of ATIV.

These effects can be interpreted as follows. We induced hypoglycemia by insulin injection and hyperglycemia by glucose injection. As is known, elevation of glucose level is always associated with increase of insulin secretion [2,6] and leads to extracellular formation of glycosylated products, which interact with nonspecific multiligand receptors (RAGE receptors) triggering the formation of free radical compounds and LPO in the cells [2,6-8,10,11]. The pancreatic Langerhans' islets have a local RAS, including a complete set of cells expressing angiotensinogen, products of its cleavage, and enzymes for its realization [2,4,10]. All these facts mean that local pancreatic RAS is largely regulated by insulin and glucose, which supports the realization of its modulatory effect on carbohydrate metabolism. Intracellular routes of signal transduction

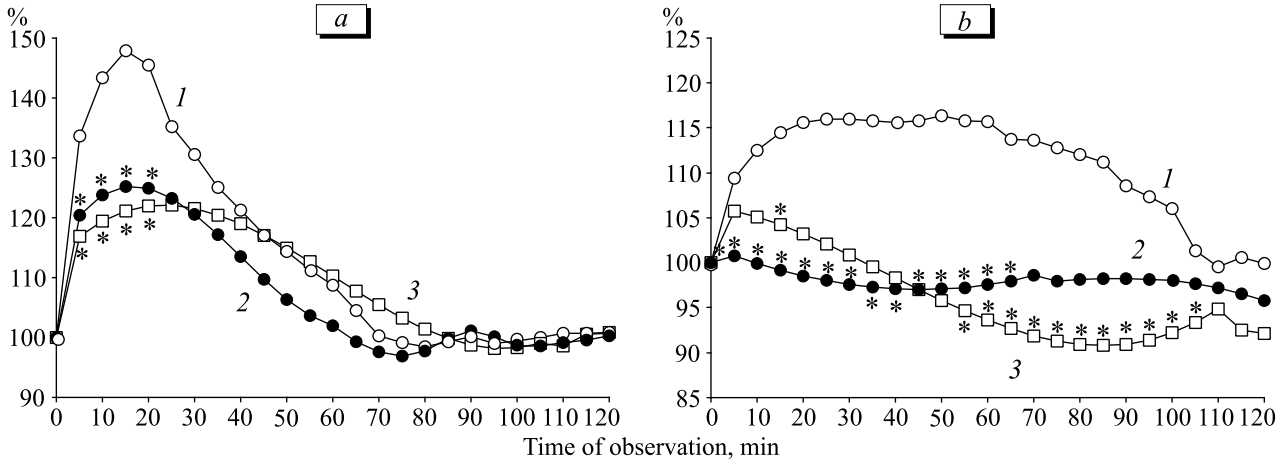

Fig. 1. Time course of SBP (*a*) and HR (*b*) in rats injected with ATII under conditions of acute hypo- and hyperglycemia. Here and in Fig. 2: ordinate: percent of basal value (100%). *1*) control (saline and ATII); *2*) hypoglycemia (insulin and ATII); *3*) hyperglycemia (glucose and ATII). *$p<0.05$ in comparison with the control.

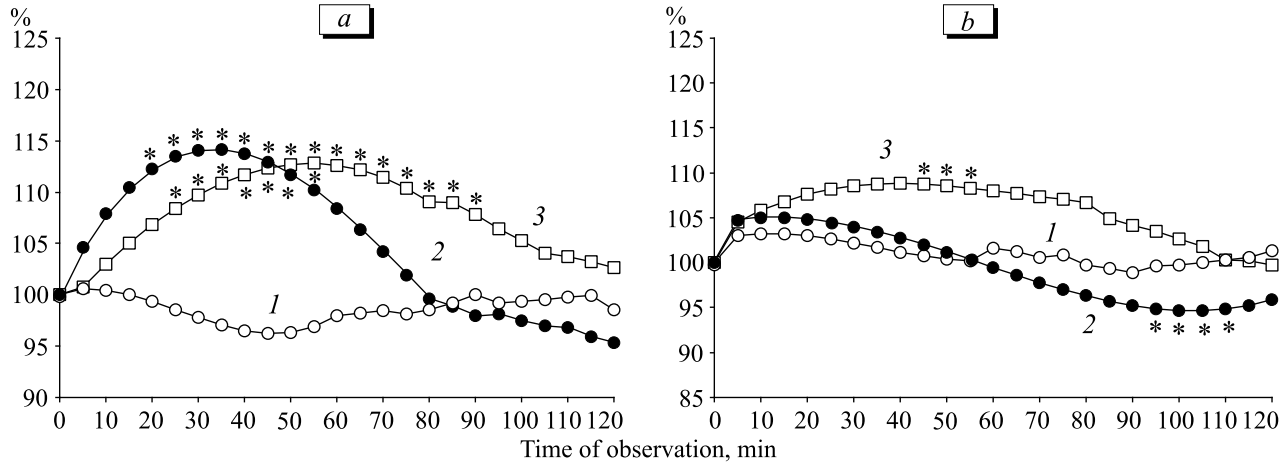

Fig. 2. Time course of SBP (*a*) and HR (*b*) in rats injected with ATIV under conditions of acute hypo- and hyperglycemia.

of ATII, glucose, and insulin, providing the formation of free radical compounds, are also mutually related [2,4,7,9,10,13]. Hence, exogenous ATII stimulates the formation of these compounds and triggers the mechanisms of desensitization of specific ATII receptors [1,3,7,9,10,13], this underlying the reduction of ATII physiological activity.

Specific ATIV receptors are insulin-dependent aminopeptidase [1,3,14,15]. Binding of ATIV to these receptors inhibits their aminopeptidase activity and increases glucose content in a cell at the expense of intracellular co-localization with glucotransporters (GLUT4). One more result of this process is more intense formation of free radical compounds, eventually providing the ATII-like effect [2,11-13].

Hence, the adaptation and compensatory processes in the RAS under conditions of shifted homeostatic constants (acute hypo- and hyperglycemia) manifest by urgent reintegration and redistribution of functions of its individual peptide components (ATII and ATIV) in metabolism regulation. This causes restructuring of the mechanisms of intra- and intersystems organization of physiological functions under extreme conditions.

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