BIOGERONTOLOGY

Peptide Correction of Age-Related Hormonal Dysfunction of the Pancreas in Monkeys

N. D. Goncharova, A. A. Vengerin, V. Kh. Khavinson*, and B. A. Lapin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 7, pp. 94-97, July, 2004
Original article submitted January 25, 2004

We studied the effect of Epithalon on the function of pancreatic islets and regulation of blood glucose level in female rhesus monkeys of various ages. Epithalon corrected the age-related decrease in glucose tolerance and restored the dynamics of insulin level in response to glucose load.

Key Words: *aging; pancreas; Epithalon; monkeys*

Clinical studies showed that physiological aging is accompanied by dysfunction of the pancreas and disturbances in glucose metabolism, which are manifested in increased basal glucose and insulin concentrations in the blood, insulin resistance, and decreased glucose tolerance [4-6]. Several endogenous and exogenous factors promote the development of these disorders. They include excessive body weight, peculiarities of fat distribution in the organism (abdominal obesity), low physical activity, and age-related neuroendocrine disorders [2,4]. Insulin resistance is the major risk factor for non-insulin-dependent diabetes mellitus (NIDDM). The morbidity rate of patients with NIDDM constantly increases. Much attention is given to the study of the mechanisms of age-related insulin resistance and synthesis of new medicinal preparations for the correction of this disorder.

Here we studied the effect of tetrapeptide Epithalon synthesized at the St. Petersburg Institute of Bioregulation and Gerontology on functional activity of pancreatic islets and glucose metabolism in rhesus monkeys (*Macaca mulatta*). Our previous studies showed that Epithalon stimulates melatonin production, which decreases with age [3]. It should be emphasized that age-related disturbances in the endocrine function of the pancreas and glucose metabolism are similar in rhesus monkeys and humans [2].

Institute of Medical Primatology, Russian Academy of Medical Sciences, Sochi; *St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences

MATERIALS AND METHODS

Experiments were performed on 7 adult young (6-8 years) and 7 old (20-27 years) clinically healthy female rhesus monkeys (*Macaca mulatta*) kept in a nursery of the Institute of Medical Primatology (Sochi-Adler). The animals were housed in individual metabolic cages (isolated room) at 20-25°C and controlled light/dark cycle (daytime 6.00-18.00). They received a balanced diet and were adapted to metabolic cages and procedure of blood sampling for no less than 4 weeks.

The glucose tolerance test was conducted 1 month before Epithalon treatment to study functional activity of pancreatic islets and glucose metabolism under basal conditions. Fasting animals intravenously received 40% glucose solution (300 mg/kg) at 9.00-9.30. The blood was sampled before and 5, 15, 30, 60, and 90 min after glucose administration.

The peptide Ala-Glu-Asp-Gly (Epithalon) was injected intramuscularly in a daily dose of 10 μg for 10

0007-4888/04/1381-0080

days (1 month after the glucose tolerance test). The glucose tolerance test was repeated on days 8-9 of treatment and 1 and 2 months after Epithalon withdrawal.

Plasma glucose concentration was measured by the glucose oxidase method. Insulin level was estimated by enzyme immunoassay with DSL kits. To estimate glucose tolerance, we measured the rate of disappearance of etogenous glucose from the blood over 15 min after glucose administration (percent per min, % per min). The results were analyzed by Student's *t* test.

RESULTS

In old monkeys the basal glucose level and its concentration 5, 15, 30, and 60 min after administration of glucose in the standard dose were higher than in young animals (Fig. 1, Table 1). Moreover, in old monkeys the basal insulin level and its concentration 30 and 60 min after glucose administration were higher than in young animals (Fig. 2). Insulin concentration in the blood of old animals significantly decreased 5 min after glucose administration (Fig. 2).

In old monkeys under basal conditions, the area under the glucose response curve (after administration of exogenous glucose in the standard dose) far surpassed that in young animals (Table 1). The rate of glucose disappearance in old monkeys was lower than in young animals (4.3±0.1 and 5.3±0.05% per min, respectively, $p<0.001$).

In old monkeys Epithalon tended to decrease the basal glucose concentration (3.8±0.4 *vs*. 4.0±0.4 mmol/liter in the control) and modulated the dynamics of glucose concentration (Fig. 1, Table 1). Glucose concentration in old animals underwent significant changes 5, 15, and 60 min after Epithalon administration. However, the concentration of glucose under basal conditions and after treatment with exogenous glucose remained practically unchanged in young monkeys (Fig. 1, Table 1).

After treatment with Epithalon the area under the glucose concentration curve after administration of exogenous glucose in the standard dose slightly decreased in old monkeys (388.9±43.6 *vs*. 479.6±38.0 mmol/liter×min in the control). Age-related differences in the area under the glucose response curve were observed under basal conditions, but disappeared after Epithalon treatment (Table 1). After Epithalon administration the rate of glucose disappearance significantly increased in old monkeys (4.92±0.20 *vs*. 4.3± 0.1% per min in the control, $p<0.01$), but remained

TABLE 1. Glucose Concentration and Area under Glucose Response Curve in Female Rhesus Monkeys of Various Ages Receiving Glucose in the Standard Dose before, during, and 1 or 2 Months after Treatment with Epithalon (*M*±*m*)

Conditions, age group	Glucose concentration, mmol/liter						Response area, mmol/liter×min
	before glucose administration	after administration of glucose in the standard dose					
		5	15	30	60	90	
Before Epithalon administration							
6-8 years	3.8±0.1	9.2±0.4	5.6±0.2	3.9±0.4	3.4±0.1	3.5±0.2	294.9±9.3
20-27 years	4.0±0.4	12.0±0.5***	9.8±0.6*	7.8±0.9*	5.0±0.4*	4.1±0.5	479.6±38.0*
During Epithalon administration							
6-8 years	3.6±0.3	8.9±0.7	6.1±0.6	3.9±0.6	3.8±0.3	3.7±0.5	343.3±48.2
20-27 years	3.8±0.4	8.4±0.6^{+}	6.8±0.8^{++}	5.7±0.9	3.9±0.4^{+++}	3.1±0.2	388.9±43.6
After Epithalon withdrawal							
1 months							
6-8 years	3.8±0.2	8.2±0.3	7.2±0.6	4.9±0.5	3.4±0.1	4.1±0.1	353.0±19.9
20-27 years	4.1±0.3	9.5±0.7^{+}	8.4±0.8	7.7±0.6**	5.2±0.5**	4.5±0.7	480.0±55.0***
2 months							
6-8 years	3.7±0.3	8.4±1.1	5.9±0.5	4.1±0.56	3.2±0.17	3.1±0.1	293.2±25.0
20-27 years	4.2±0.4	8.6±0.7^{+++}	8.1±0.6**$^{+++}$	7.4±1.0**	5.3±0.9***	4.1±0.6	451.0±46.0**

Note. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to 6-8-year-old animals; $^{+}p<0.001$, $^{++}p<0.01$, and $^{+++}p<0.05$ compared to indexes observed before Epithalon administration.

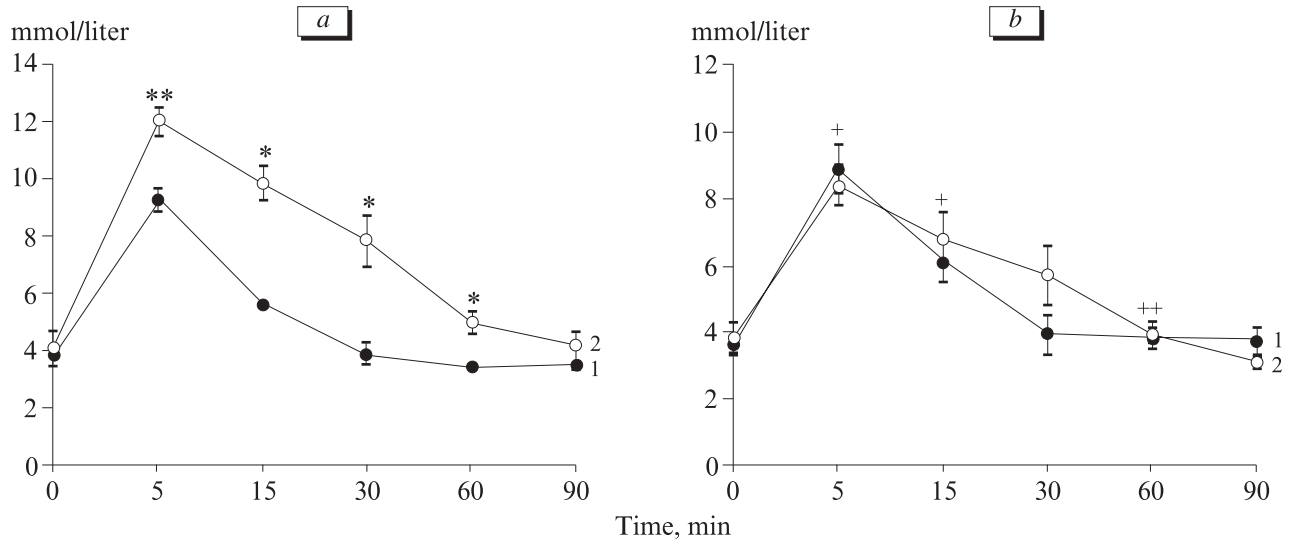

Fig. 1. Glucose concentration in peripheral blood plasma from young (*1*) and old female rhesus monkeys (*2*) receiving glucose in the standard dose before (*a*) and on days 8-9 of Epithalon treatment (*b*). **p*<0.001 and ***p*<0.05 compared to young animals; ^{+}p<0.001 and ^{++}p<0.05 compared to indexes observed before Epithalon administration.

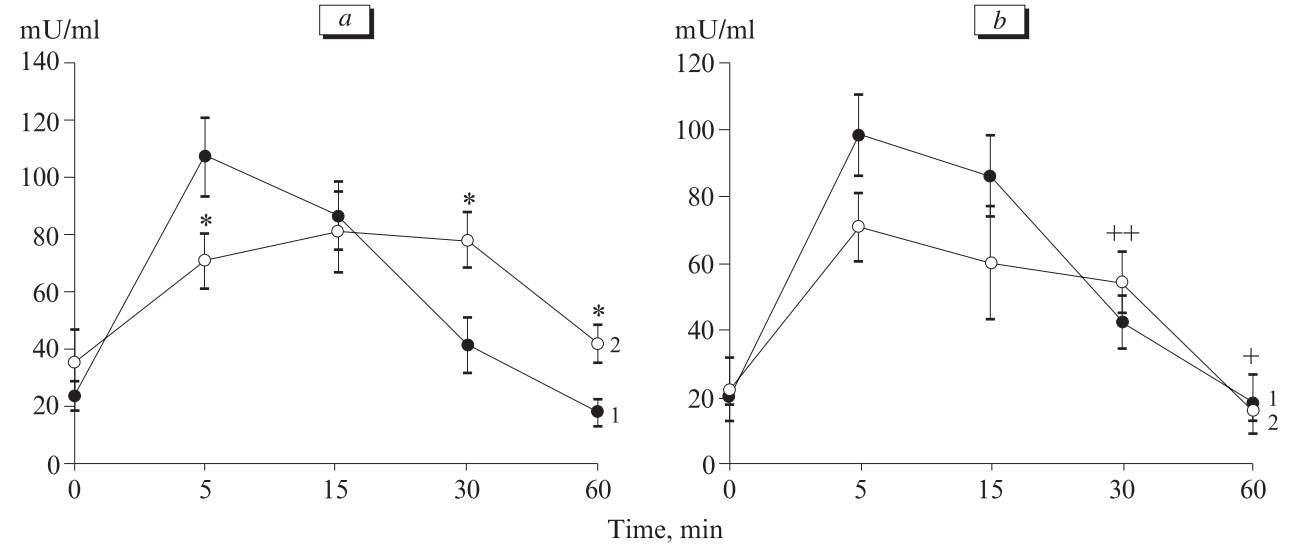

Fig. 2. Insulin concentration in peripheral blood plasma from young (*1*) and old female rhesus monkeys (*2*) receiving glucose in the standard dose before (*a*) and on days 8-9 of Epithalon treatment (*b*). **p*<0.05 compared to young animals; ^{+}p<0.001 and ^{++}p<0.05 compared to indexes observed before Epithalon administration.

unchanged in young animals (5.20±0.18 *vs* 5.20±0.05 % per min in the control, $p>0.05$).

Basal glucose concentration, its level 30, 60, and 90 min after glucose administration, and area under the glucose response curve returned to normal 1 and 2 months after Epithalon withdrawal (Table 1). However, 5 and 15 min after treatment glucose concentration remained below the basal level (Table 1). Moreover, the rate of glucose disappearance 1 and 2 months after Epithalon withdrawal was higher than in the control (4.7± 0.3, 4.96±0.20, and 4.3±0.1% per min, respectively).

Basal insulin concentration decreased after Epithalon administration. Glucose-induced changes in insulin concentration were similar in old and young monkeys (Fig. 2). In old monkeys receiving Epithalon the relative increase in insulin concentration became more pronounced 5 min after glucose administration (320±29 *vs*. 198±40% in the control, $p<0.05$; in young animals 450±72%), but decreased by the 60th minute (69±8 *vs*. 117±20% in the control, $p<0.05$).

In old monkeys, insulin concentration increased, while glucose level decreased 5 min after administration of glucose in the standard dose. These changes indicate that Epithalon normalizes phase I of insulin secretion associated with a rapidly reacting pool. Probably, the sensitivity β-cells of Langerhans islets to

glucose in high concentrations increases. Disturbances in the early phase of insulin secretion were observed in patients with NIDDM and impaired glucose tolerance [1].

Epithalon not only normalized the early phase of insulin secretion, but also increased "plasticity" of phase II. Insulin concentration significantly decreased 30 and 60 min after glucose administration (Fig. 2). Normalization of insulin level in old monkeys was decelerated under basal conditions (Fig. 2), which is consistent with published data [2]. Insulin concentration returned to normal after 30 and 60 min, which is probably related to an increase in the sensitivity of peripheral tissues to insulin. This assumption is confirmed by a decrease in insulin and glucose concentrations (Table 1, Fig. 1).

Our results indicate that treatment with Epithalon for 10 days normalizes impaired glucose tolerance and corrects changes in blood insulin level in aging monkeys. Glucose tolerance remained high 1 and 2 months after Epithalon withdrawal. Epithalon produces a normalizing effect on functional activity of pancreatic islets and glucose metabolism. Probably, Epithalon restores sensitivity of β-cells and peripheral tissues to blood glucose and insulin, respectively. It can be hypothesized that the effect of Epithalon is realized via stimulation of melatonin secretion [3]. Previous studies showed that melatonin decreases basal insulin concentration [9], *in vitro* inhibits its production in Langerhans islets [8], and prevents hyperglycemia during experimental diabetes mellitus [7].

REFERENCES

1. A. S. Ametov, *Probl. Endokrinol*., **48**, No. 3, 31-37 (2002).
2. N. D. Goncharova, A. A. Vengerin, T. E. Oganyan, and B. A. Lapin, *Byull. Eksp. Biol. Med*., **137**, No. 3, 317-320 (2004).
3. N. D. Goncharova, V. Kh. Khavinson, and B. A. Lapin, *Ibid*., **131**, No. 4, 466-468 (2001).
4. M. Barbiery, M. R. Rizzo, D. Manzella, *et al*., *Exper. Gerontology*, **38**, Nos. 1-2, 137-143 (2002).
5. F. L. Bellino and P. M. Wise, *Biol. Reprod*., **68**, No. 1, 10-18 (2003).
6. Z. Huang, N. L. Bodkin, H. K. Ortmeyer, *et al*., *J. Clin. Invest*., **94**, No. 3, 1289-1296 (1994).
7. P. L. Montilla, J. F. Vargas, I. F. Tunez, *et al*., *J. Pineal Res*., **25**, No. 2, 94-100 (1998).
8. E. Peschke, D. Peschke, T. Hammer, and V. Csernus, *Ibid*., **23**, No. 3, 156-163 (1997).
9. D. D. Rasmussen, B. M. Boldt, C. W. Wilkinson, *et al*., *Endocrinology*, **140**, 1009-1012 (1999).