Angiotensin II-induced motility of reservoir smooth muscle organs from ghrelin and melatonin-treated diabetic rats

P.V. Hadzhibozheva[1*], Ts. K. Georgiev[1], R. E. Kalfin[2], G. S. Ilieva[1], A. N. Tolekova[1]

[1]*Department.of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, 11 Armeiska Str., Stara Zagora 6000, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, 23 Akad. Georgi Bonchev Str., Sofia 1113, Bulgaria*

The purpose of the study was to assess the effect of short–term ghrelin (GHR) or melatonin (MLT) treatment on Angiotensin II (AngII)-provoked motility of stomach, rectum and urinary bladder of rats with streptozotocin (STZ)-induced diabetes. Mature Wistar rats were divided into 4 groups: control; STZ-treated: by a single STZ injection; MLT-treated: single STZ injection, followed by MLT treatment for 7 consecutive days; GHR-treated: single STZ injection, followed by GHR treatment for 7 consecutive days. The experiment lasted 42 days and in the end, preparations from the reservoir organs were prepared and influenced by AngII. The analysis of power and kinetic parameters of the obtained contractions was made by KORELIA Software.

STZ-induced diabetes affected differently AngII-provoked contractile activity of reservoir organs. In the MLT-treated group, powerful responses to Ang II of the stomach (1.91 ± 0.07 g) and weak Ang II-induced contractions of urinary bladder preparations (1.12 ± 0.11 g) in comparison to controls (1.14 ± 0.13g and 1.74 ± 0.22g, respectively) were observed. Administration of GHR almost completely recovered the normal force characteristics of urinary bladder contractions and accelerated the duration of stomach contractions. The responses to Ang II of rectal preparations from animals treated with GHR or MLT were not improved.

Although partial, there were registered favorable effects of short-term application of MLT or GHR in animals with STZ-induced diabetes. The beneficial effect on Ang II-induced stomach and urinary bladder motility was probably due to antioxidant and pro-kinetic properties of MLT or GHR on the smooth muscle.

Key words: Angiotensin II, Ghrelin, Melatonin, diabetes, smooth muscle

INTRODUCTION

The stomach, rectum and urinary bladder serve mainly as reservoir organs and perform evacuating functions. This is why the maintenance of their adequate tone is essential for a normal quality of life. The precisely coordinated and complex smooth muscle activity of reservoir organs is regulated by the interplay between neural and endocrine control mechanisms. The octapeptide Angiotensin II (Ang II) is an important factor for blood pressure regulation and maintenance of electrolyte homeostasis. Furthermore, as the main effector of the Renin-Angiotensin System (RAS), Ang II has various actions, many of them affecting the activity of visceral smooth muscles from gastrointestinal (GI) and urogenital tract [1,2]. Leung et al. [3] have found that Ang II has a potent contractile action on the musculature of the GI tract, rather than on the aorta. In the GI tract, Ang II plays multiple roles, influencing water-salt balance, blood flow, motility and inflammation. There is evidence for the involvement of Ang II in the development of gastro-esophageal reflux [2], internal anal sphincter

incontinence [4] and Crohn's disease [2,5]. It has also been shown that Ang II causes dose-dependent contractions of smooth muscle strips from the urinary bladder. This directs many researchers to the hypothesis that Ang II is likely to influence the process of micturition and probably acts as a modulator of neurotransmission in the bladder [6]. A large number of studies reveal the presence of receptors for Ang II in different parts of the GI and urogenital tracts [2,5]. Most of the effects of Ang II on the digestive system, especially those concerning the contractile activity, are attributed mainly to the effects mediated by the AT1 receptors [2,4,6].

Nowadays, the growing incidence of disorders in many smooth muscle organs is frequently observed in diabetic patients. The gastroparesis, fecal incontinence/constipation and cystopathy are among the first significant complications connected with the progression of diabetes mellitus [7,8]. The reason for these diabetic complications is the impaired smooth muscle function due to oxidative stress and the accumulation of glycated products [9]. Considering the leading role of oxidative stress in the pathogenesis of diabetes, the scientific efforts are directed to the search for effective antioxidants [10]. Such possible antioxidants with a therapeutic

* To whom all correspondence should be sent:
E-mail: petia_hadjibojeva@abv.bg

potential for treatment of diabetic smooth muscle dysfunction could be the hormones melatonin (MLT) and ghrelin (GHR).

Melatonin (MLT)

MLT positively affects a wide range of diabetic complications by reducing the oxidative stress. It has been found that MLT is a more powerful antioxidant than Vitamin E, displaying nearly 10-fold times more potent free radicals trapping ability, especially in the brain [11]. An intraperitoneal injection of MLT, made a few days before STZ-induced diabetes in rats, prevents severe lesions of β-cells in the pancreas [12]. Klepac et al. [13] found that even a single dose of MLT (20 mg / kg) has an antioxidant activity in the plasma of STZ-treated rats, increases the action of antioxidant enzymes and reduces the production of superoxide radicals.

Ghrelin (GHR)

Irako et al. [14] are the first who found that a subcutaneous injection of GHR could prevent the hyperglycemia, caused by the STZ application in newborn rats. The authors have registered a significant increase in insulin production and secretion in the experimental animals [14]. In similar experiments, Granata et al. [15] described that the administration of GHR results in an improvement of glucose metabolism and a conservation of mass of pancreatic island cells. This is a prerequisite for a good therapeutic potential of GHR in conditions associated with an impaired β-cell function [15]. GHR favorably affects the gastropathy, stimulates the motility and emptying of the stomach and accelerates the delayed by the diabetes intestinal passage [16-18].

The established role of RAS in the pathogenesis of hypertension in diabetes mellitus [19] focused our interest to study the effects of Ang II on diabetic visceral smooth muscles. Despite the observed development of diabetic smooth muscle dysfunction in a number of organs, the information about the changes in the smooth muscle response of reservoir organs to Ang II, in this disease, is insufficient. The hormones MLT and GHR are with proven protective and antioxidant effects and possess a promising action in the prevention of the emergence and development of diabetic complications. Currently, there is no information in the literature how the application of these hormones could influence Ang II - stimulated responses of diabetic visceral smooth musculature.

The aim of this study was to assess whether short–term application of MLT or GHR on rats with streptozotocin (STZ)-induced diabetes will affect the Ang II - induced motility of the stomach, rectum and urinary bladder.

EXPERIMENTAL

Experimental animals

Mature Wistar rats, weighting 250-300 g, were divided into 4 groups: control group – healthy rats, injected 8 consecutive days from the beginning of the experiment with saline; STZ-treated group (diabetic group) - rats injected once on the first day of the experiment with a single dose of STZ; MLT-treated group (diabetic animals treated with MLT) - rats injected once on the first day of the experiment with a single dose of STZ, followed by 7 consecutive days administration of MLT; GHR-treated group (diabetic animals treated with GHR) - rats injected once on the first day of the experiment with a single dose of STZ, followed by 7 consecutive days administration of GHR.

Experimental model of diabetes mellitus and treatment with MLT and GHR

The induction of diabetes was made by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg. STZ was dissolved in cold 0.1 M citrate buffer, pH 4.5. The injected volume did not exceed 0.1 ml in each experimental animal. 72 hours after STZ application (the third day of the experiment), blood glucose levels were measured and only animals with blood glucose above 16 mmol/l were considered diabetic and were left in the experiment.

MLT was administered in the diabetic animals at a dose of 10 mg / kg i.p. This dosage and the route of administration of MLT were made as it was described by some authors [20,21]. GHR was administered in the diabetic animals at a dose of 100 μg/kg s.c. This dosage and the route of administration of GHR were made as it was described by Irako et al. [14] and Granata et al. [15].

The experiment lasted 42 days and in the end, preparations from the reservoir organs were made and influenced by Ang II.

Sample Preparation

The study was performed on stomach, rectal and urinary bladder smooth muscles, isolated from the experimental animals. The animals were anesthetized with Nembutal 50mg/kg i.p. and exsanguinated. The experiments were carried out in accordance with the National regulations and the Directive 2010/63/EU of the European parliament and of the Council (22 September, 2010)

concerning the protection of animals used for scientific purposes.

Abdominal and pelvic cavity were opened and the stomach, rectum and urinary bladder were dissected out and immediately placed in cold Krebs solution (3 °C), containing the following composition (in mmol): NaCl 118.0, KCl 4.74, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, $CaCl_2$ 2.0, KH_2PO_4 1.2, and glucose 11.0. The surrounding tissue was dissected and longitudinal sections from the organs (approximately 8-10 mm long) were prepared. The two ends of each preparation were tied with ligatures. The distal end was connected to the organ holder; the proximal end was stretched and attached to a mechano-electrical transducer FSG-01 (Experimetria Ltd., Hungary) via a hook. The preparations were placed in organ baths TSZ-04/01 (Experimetria Ltd., Hungary), containing Krebs solution, pH 7.4, continuously bubbled with Carbogen (95% O_2, 5% CO_2). The organ baths were mounted in parallel above an enclosed water bath, maintaining the solution temperature at 37 °C. Preparations were placed under an initial tension (preload) of 1 g and allowed to equilibrate for at least 75 min (three periods: 15 min, 45 min and 15 min and two washes with Krebs solution between them). After the equilibration period, preparations were influenced by Ang II in a dose of 1 μmol (10^{-6} M).

Recording of mechanical activity and technical equipment

Mechanical activity was digitized and recorded by using ISOSYS-Advanced 1.0 Software (Experimetria Ltd., Hungary). Data processing and storage for subsequent analysis were performed with specialized software KORELIA [22]. With the module KORELIA-Processing [23] a transformation of data from ISOSYS-Advanced 1.0 was performed and their primary processing (filtering, smoothing, scaling, etc) was made.

Chemicals and drugs

Ang II (Sigma-Aldrich, Germany) was solubilized in bidistilled water. STZ and all reagents for the preparation of Krebs solution were purchased from Sigma-Aldrich Chemie GmbH, Germany. MLT (Sigma-Aldrich, Germany) and was dissolved in 1:90 ethanol/saline immediately prior to injection of the experimental animals. GHR (PolyPeptide Group, Sweden) was dissolved in saline immediately prior to injection of the experimental animals.

Data processing

The duration of the interval for analysis of Ang II - induced smooth muscle contractions was defined as follows: from the beginning of the contraction, until the moment at which the amplitude dropped to 50% of its maximum. This definition was made in order to calculate uniformly the various in duration contractions (Fig.1).
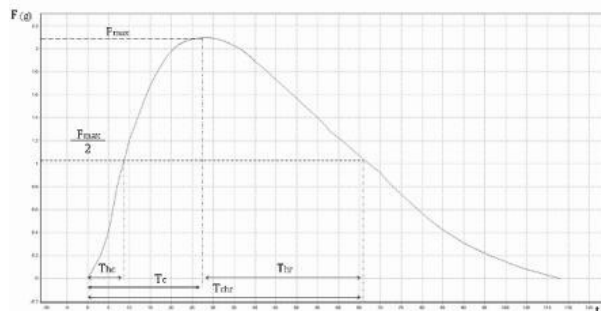

Fig. 1. Time-parameters of Ang II-induced contraction: F_{max} – maximal force of the smooth muscle contraction (SMC), $F_{max}/2$ – half of maximal force of the SMC, T_{hc} – half-contraction time: the time interval between the start of the SMC and $F_{max}/2$, T_c – contraction time: time interval between the start of the SMC and F_{max} , T_{hr} – half-relaxation time: the interval between F_{max} and $F_{max}/2$, T_{chr} – contraction plus half-relaxation time: the interval between the beginning of the SMC until the amplitude fell to $F_{max}/2$

The recorded force-vs.-time curves allowed the determination of the amplitude and the integral force of the contraction (the latter represented by the area under the curve - AUC). The different phases of the Ang II - induced tonic contractions, were clarified and analyzed by the application of a time -parameter analysis, similar to that made in the study of the skeletal muscle contraction [24]. The following time-parameters [25] were defined (Fig.1): half-contraction time (T_{hc}), contraction time (T_c), half-relaxation time (T_{hr}), contraction plus half-relaxation time (T_{chr}). Their calculation was made by KORELIA-Dynamics Program [26]. The averaged time-parameters were processed by spline interpolation and graphical visualization of the different patterns of contractile activity was obtained.

Statistical analysis

Obtained data were processed by the statistical program Statistica Version 6.1 (StaSoft, Inc., Tulsa, OK, USA) and presented as a mean ± standard error. A P-value less than or equal to 0.05 was considered to be statistically significant.

RESULTS

Stomach

The comparison in force parameters of Ang II-induced contractions (Fig.2) showed that the stomach preparations of the MLT-treated group developed the strongest answer to Ang II (amplitude and AUC respectively 1.99 ± 0.07 g and 315.65 ± 25.50 gs). The AUC of the contractions of the other three groups did not differ statistically ($P> 0.05$). Ang II-induced contractions of the stomach preparations from the STZ-treated group were with similar force parameters as those of the control group (Fig.2).

Time-parameters analysis revealed that the developed response to Ang II of the preparations from the diabetic group was faster and all the parameters were significantly shortened compared to those of control, with the exception of T_c (Table 1).
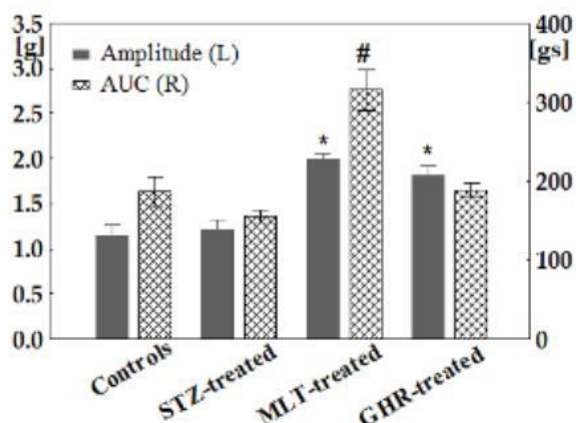
Fig. 2. Amplitude and AUC of Ang II - induced contractions of gastric preparations from the different groups. ******P* < 0.05 vs amplitudes of contraction of gastric preparations of controls and STZ-treated group [#] *P* < 0.05 vs AUC of contraction of gastric preparations of controls, STZ- and MLT-treated animals.

The contractions of the preparations from the GHR-treated group were the fastest, with most shortened time-parameters (Table 1). This pattern of contractile activity was clearly visualized when interpolation was performed (Fig.5-A). Graphical visualization of Ang II - induced activity of stomach preparations of the different groups revealed the similarity between the contractions of preparations from MLT-treated and control groups (Fig.5-A).

Rectum

The responses to Ang II of rectal preparations of STZ-, MLT- and GHR-treated animals were with similar, significantly lower amplitude, when compared to controls (Fig.3). The latter developed the most powerful contraction: AUC 332.71 ± 35.78 gs, while the AUC of preparations from the GHR-treated group was significantly reduced: 143.17 ± 18.69 gs (Fig.3).

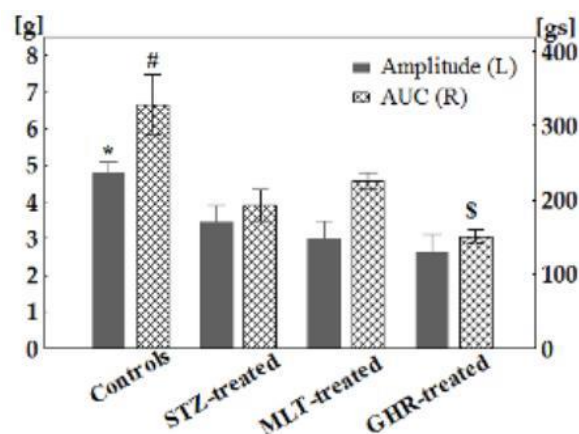**Fig.3.** Amplitude and AUC of Ang II - induced contractions of rectal preparations from the different groups. ******P* < 0.05 vs amplitudes of contraction of rectal preparations from STZ-, MLT- and GHR-treated animals. [#] *P* < 0.05 vs AUC of contraction of rectal preparations of STZ-, MLT- and GHR-treated animals. [$] *P* < 0.05 vs AUC of contraction of rectal preparations of controls, STZ- and MLT-treated animals.

Table 1. Calculated time – parameters of Ang II – induced stomach contractions from the different groups.

Group	T_{hc} (s)	T_c (s)	T_{hr} (s)	T_{chr} (s)
Controls	28.69 ± 2.53	78.18 ± 3.35	146.73 ± 9.84	224.91 ± 11.64
STZ-treated	18.00 ± 0.96	77.75 ± 1.69	105.50 ± 5.18	183.25 ± 4.98
MLT-treated	17.20 ± 1.02	66.00 ± 0.71	157.01 ± 1.41	222.81 ± 2.11
GHR-treated	27.33 ± 4.84	63.66 ± 6.97	94.00 ± 7.10	157.67 ± 4.59

Table 2. Calculated time – parameters of Ang II – induced rectal contractions from the different groups.

Group	T_{hc} (s)	T_c (s)	T_{hr} (s)	T_{chr} (s)
Controls	9.58 ± 1.52	39.74 ± 3.23	48.43 ± 5.74	87.86 ± 7.83
STZ-treated	13.50 ± 0.92	44.33 ± 4.83	50.50 ± 4.14	94.83 ± 5.73
MLT-treated	12.17 ± 1.40	42.32 ± 3.95	72.67 ± 2.01	115.02 ± 5.77
GHR-treated	8.33 ± 0.76	29.00 ± 2.98	53.17 ± 5.66	82.17 ± 6.44

The time-parameters analysis (Table 2) did not reveal significant differences between the contractions of the preparations from the control and the STZ-treated group.

This similarity in the response to Ang II of these two groups was further observed when graphic images of contractions were performed (Fig.5-B): the two patterns of Ang II-induced activity differed only in force parameters.

Interestingly, the Ang II – provoked contractions of the rectal preparations from the GHR-treated group, also displayed similar duration in time, while the contractions of MLT-treated rats were significantly prolonged, with increased T_{hr} and T_{chr} (Table 2, Fig.5-B).

Urinary bladder

The application of Ang II on urinary bladder preparations from control and GHR-treated group caused contractions with similar force parameters: amplitude 1.74 ± 0.22 g and 1.63 ± 0.19 g, respectively and AUC 121.13 ± 13.73 gs and 121.11 ± 6.96 gs, respectively.
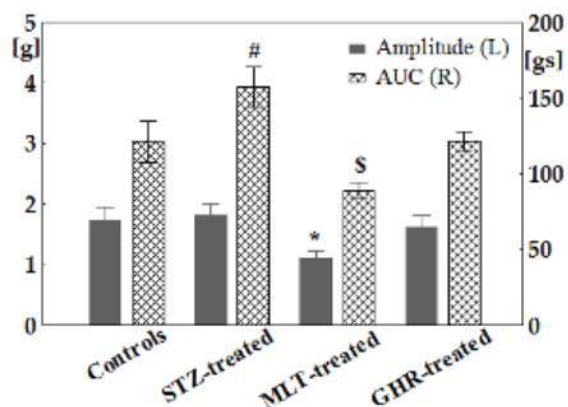

Fig. 4. Amplitude and AUC of Ang II - induced contractions of urinary bladder preparations from the different groups. ***P* < 0.05 vs amplitudes of contraction of bladder preparations from controls, STZ- and GHR-treated animals. # *P* < 0.05 vs AUC of contraction of bladder preparations of controls, MLT- and GHR-treated animals. $ *P* < 0.05 vs AUC of contraction of bladder preparations of controls, STZ- and GHR-treated animals.

The amplitude of Ang II-induced response of preparations from STZ-treated rats $(1.81 \pm 0.18$ g) did not differ statistically from the above, but the integral force was significantly increased $(157.43 \pm 13.13$ gs). Preparations from MLT-treated group displayed the weakest reaction to Ang II (amplitude 1.12 ± 0.11g and AUC 88.93 ± 5.40 gs) (Fig.4).
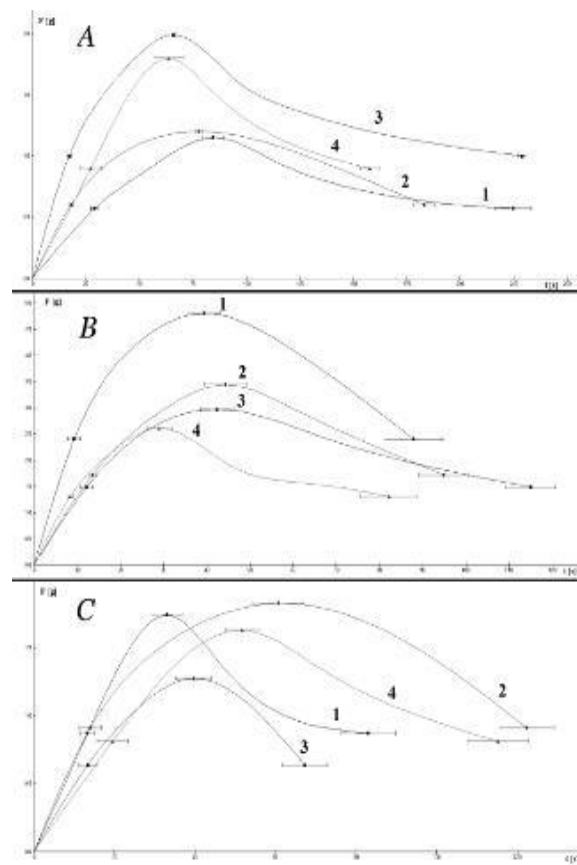

Fig. 5. Graphic images of Ang II - induced activity of smooth muscle preparations from: healthy animals: control group (**1**); diabetic animals: STZ-treated group (**2**); diabetic animals, treated additionally with MLT: MLT-treated group (**3**); diabetic animals, treated additionally with GHR: GHR-treated group (**4**).
A. Preparations of stomach ,**B.** Preparations of rectum, **C.** Preparations of urinary bladder

The comparison of the contractions by time-parameters (Table 3) showed that Ang II-induced responses of the four groups had similarity in reaching T_{hc}. T_c and T_{chr} however, were significantly prolonged in the contractions of preparations from STZ-treated and GHR-treated groups, while T_{hr} of preparations from MLT-treated animals was shortened (Table 3).

The graphical visualization of the contractile process (Fig.5-C) showed different models of Ang II-provoked urinary bladder activity in the different groups.

There were observed similarities between the contractile patterns of STZ-treated and GHR-treated groups from one side, and control and MLT-treated group from another.

Table 3. Calculated time – parameters of Ang II – induced urinary bladder contractions from the different groups.

Group	T_{hc} (s)	T_c (s)	T_{hr} (s)	T_{chr} (s)
Controls	13.28 ± 1.69	33.00 ± 3.14	50.28 ± 5.14	83.00 ± 6.95
STZ-treated	14.00 ± 2.78	60.71 ± 8.46	61.71 ± 6.58	122.43 ± 7.89
MLT-treated	13.33 ± 1.45	39.67 ± 4.59	30.60 ± 2.66	67.33 ± 3.88
GHR-treated	19.50 ± 4.88	51.67 ± 7.14	63.67 ± 4.94	115.33 ± 7.98

DISCUSSION

STZ-induced diabetes and smooth muscle dysfunction of reservoir organs

The similarity in force characteristics of the responses to Ang II of stomach and urinary bladder preparations from control and STZ-treated group, respectively, show that probably 6-week period after administration of STZ was not sufficient to reveal the characteristic changes in the function of these organs. Some authors also do not establish any differences between cholinergic responses of such preparations from healthy rats and rats with STZ-induced diabetes, lasted 6-8 weeks [27,28]. It is likely that this lack of apparent differences in the contractile response of preparations from healthy and diabetic animals to be due to not yet occurred or compensated changes in the smooth musculature.

However, we observed an accelerated time for contraction (T_{hc}) and relaxation (T_{hr}) of Ang II – induced gastric reaction in STZ-treated animals. This is an evidence for a more rapid duration of Ang II – stimulated contractile process in the stomach during diabetes. Such increased motor function of the stomach in diabetic patients has already been described [29,30]. It is believed that this dysfunction is caused by the hyperglycemia and the subsequent neuropathy, damage of gastric pacemaker cells and myopathy [29]. Probably this accelerated gastric emptying is a preliminary stage of the later observed delayed stomach evacuation [30]. According to He et al. [31], Ang II also takes part in the pathogenesis of the diabetic gastropathy. The authors reported that in patients with diabetes, the activity of RAS and Ang II, in particular, are increased. In a study of Tobu et al. [32] on rats with STZ-induced diabetes, an increased expression of AT1 receptors in smooth muscle cells of the urinary bladder was described. The authors believe that the persistent hyperglycemia probably activates the local RAS in the bladder.

The contractions of bladder preparations from STZ-treated animals were with delayed contraction time (T_c), which led to a prolonged development of smooth muscle process and an increased AUC. Such an increased activity of the bladder in STZ-treated animals might be associated with increased release of neurotransmitters, improved activity of Ca^{2+}-channels or increased calcium sensitivity [28].

The analysis of rectal contractions of STZ-treated animals found exactly the opposite response to Ang II: a significant reduction of force parameters. This is consistent with Touw et al. [33], who reported for a less motor activity of large intestine in diabetic mice. In diabetes, there is a reduced entry of Ca^{2+} in smooth muscle cells of the rectum, resulting in decreased and disturbed contractile activity. Furthermore, Jancsó et al. [34] have found that the large intestine is more sensitive to the oxidative stress, caused by diabetes, when compared to the small intestine. The primary defect that leads to a less rise in intracellular Ca^{2+} concentration is a violation of L-type Ca^{2+}-channels, probably due to accumulation of glycated products [33].

Effect of MLT treatment

The fact, that contractions of the preparations from MLT-treated group differed when compared to controls and STZ-treated ones, revealed that: 1) short-term administration of MLT at the beginning of the experiment was not enough to influence entirely the smooth muscle dysfunction, caused by diabetes; 2) yet, there was some effect of the application of MLT in the diabetic animals.

The registered decreased response to Ang II of the urinary bladder from MLT-treated group is probably due to influence on the mechanism of contraction. There is evidence that MLT inhibits Ca^{2+}-calmodulin complex and directly affects the ion channels on the urinary bladder smooth muscle cells, thus preventing the contractile process [35]. A similar type of MLT action could be suggested for the rectal preparations, where Ang II-provoked contractions were also with reduced force characteristics.

Regarding the stomach, the observed differences between the responses to Ang II of preparations from STZ- and MLT-treated groups indicate a beneficial effect of short-term MLT application. Even though the force parameters were increased, the time-parameters of gastric contractions from MLT-treated group were nearly identical to the controls. It is known that, due to its antioxidant properties, MLT stimulates the immune system,

improves the microcirculation and epithelial regeneration, and thus protects the digestive tract [36]. According to Peschke [37], MLT can significantly reduce the levels of protein glycation. Considering the role of ROS and glycation end products for the impaired smooth muscle activity in diabetes, it can be assumed that the favorable effect on the duration of stomach contraction is due to all the mentioned above properties of MLT. It should be also taken into account, that the disorders of the intestinal and gastric function during the diabetes do not show correlation between each other. For example, disturbances in the upper GI tract may occur in later stages of the disease, compared to the large intestine [38].

Effect of GHR treatment

In our experiments, we found differences between Ang II - stimulated contractions of the preparations from GHR-treated group, and the other groups. This indicates that short-term application of GHR had effect on the STZ-induced diabetes. The diverse contractile pattern of the stomach, rectum and bladder revealed a different influence of GHR on the diabetic smooth muscle activity.

The registered rapid response to Ang II of gastric preparations from GHR-treated group was in agreement with Qui et al. [17,18]. In a series of experiments with diabetic animals, these researchers have found that the treatment with GHR accelerates gastric and small intestinal contractile activity and leads to an increase in the amplitude of the carbachol-induced contractions. It is suggested that the stimulatory effect on the activity of the stomach is due to the activation of peripheral cholinergic pathways in the enteric nervous system [17,18]. GHR and its agonists also contribute to the restoration of the gastric mucosa damaged by oxidative stress in diabetes. These gastro-protective properties are likely to be due to the established antioxidant activity of the peptide [16]. In experiments with STZ-induced diabetes in rats, Ariga et al. [39] found high levels of GHR in the blood plasma of the animals and increased gastric evacuation activity. The authors suggest that during the early stages of diabetes, the elevated levels of the endogenous GHR improve the coordination between the fundus and pylorus of the stomach and accelerate the evacuation of the food. Moreover, a study of patients with a chronic heart failure reveals that serum levels of GHR correlated with Ang II levels and GHR can inhibit Ang II-induced cardiomyocyte apoptosis by down-regulating AT1 receptors [40]. Considering that during diabetes the levels of endogenous GHR are higher [39], the activity of Ang II rises [31] and the expression of AT1 is increased [32], such correlation between GHR and Ang II levels and suppression of AT1 receptors could be supposed.

The Ang II-stimulated contractions of bladder preparations from GHR- treated group showed similar force parameters, when compared to the control group. These results indicated that GHR application had a beneficial effect on the urinary bladder activity. We could assume that this effect is due to the antioxidant or the described above interaction between GHR and AT1 receptors.

Interestingly, the rectal preparations of GHR-treated animals responded to Ang II with weaker reaction. According to Zhao et al. [41], GHR can play a role as pro-inflammatory peptide in the large intestine, thus promoting the formation of inflammatory cytokines in this region of the GI tract. Taking into account that the large intestine is more sensitive to the oxidative stress, caused by the diabetes [34], probably the seven-day administration of GHR on diabetic animals have caused an additional damage in the rectum, hence affecting the Ang II-stimulated contractile activity. In support of this hypothesis is the research conducted by Liu et al. [42], who found that the activation of the GHR receptors in the large intestine contributes to the development of colitis, probably by enhancing the pro-inflammatory cytokines and activation of macrophages.

In conclusion, the seven-day administration of MLT or GHR on rats with experimentally induced diabetes mellitus had a positive effect on some parameters of the Ang II –induced response of the preparations from stomach and urinary bladder. This beneficial effect was probably due to antioxidant and pro-kinetic properties of MLT or GHR on the smooth musculature of these organs. On the other hand, the responses to Ang II of rectal preparations from animals treated with GHR or MLT were not improved. Obviously, Ang II-mediated contractile activity of the rectum is seriously impaired by the diabetes and could not be enhanced by a short-term application of these hormones.

REFERENCES

1. S.I. Shimuta, A.C. Borges, R.N. Prioste, T.B. Paiva, *Eur. J. Pharmacol.,* **367**, 59 (1999).
2. L. Fändriks. *J. Renin Angiotensin Aldosterone Syst.,* **11**, 43 (2010).
3. E. Leung, J.M. Rapp, L.K. Walsh, K.D. Zeitung, R.M. Eglen, J. *Pharmacol. Exp. Ther.*, **267**,1521 (1993).
4. S. Rattan, R.N. Puri, Y.P. Fan, *Exp. Biol. Med.,* **228**, 972 (2003).
5. G.D.Wang, X.Y.Wang, H.Z. Hu, X.C. Fang, S. Liu, N. Gao, Y. Xia, J.D. Wood, *Am. J. Physiol. Gastrointest. Liver Physiol.*, **289**, 614 (2005).
6. K. Andersson, A. Arner, *Physiol. Rev.,* **84**, 935 (2004).
7. P. Kashyap, G. Farrugia, *Neurogastroenterol. Motil.,* **23** (2), 111 (2011).
8. Frimodt-Møller, *Ann. Int. Med.,* **92**, 318 (1980).
9. M.J. Chang, J.H. Xiao, Y. Wang, Y.L. Yan, J. Yang, J.L. Wang, *PLoS One,* **7**(12), e50291 (2012).
10. A.Chatzigeorgiou, A. Halapas, K. Kalafatakis, E. Kamper, *In Vivo,* **23**, 245 (2009).
11. G. Baydas, H. Canatan, A. Turkoglu, *J Pineal Res*., **32**, 225 (2002).
12. O. Yavuz, M. Cam, N. Bukan, A. Guven, F. Silan, *Acta Histochem.*, **105**, 261 (2003).
13. N. Klepac, Z. Rudes, R. Klepac, *Biomed. Pharmacother.*, **60**, 32 (2006).
14. T. Irako, T. Akamizu, H. Hosoda, H. Iwakura, H. Ariyasu, K. Tojo, N. Tajima, K. Kangawa, *Diabetologia.*, **49**,1264 (2006).
15. R. Granata, M. Volante, F. Settanni, C. Gauna, C. Ghé, M. Annunziata, B. Deidda, I. Gesmundo, T. Abribat, A.J. van der Lely, G. Muccioli, E. Ghigo, M. Papotti, *J. Mol. Endocrinol,* **45**, 9 (2010).
16. H. Suzuki, J. Maztsizaki, T. Hibi, *J Clin. Biochem. Nutr.*, **48**, 1 (2010).
17. W.C. Qiu, Z.G. Wang, R. Lv, W.G. Wang, X.D. Han, J. Yan, Y. Wang, Q. Zheng, K.X. Ai, *World J. Gastroenterol.*, **14**, 2572 (2008)
18. W.C. Qiu, Z.G. Wang, W.G. Wang, J. Yan, Q. Zheng, *World J Gastroenterol.,***14**, 1419 (2008).
19. A. Ribeiro-Oliveira, A.I. Nogueira, R.M. Pereira , W.W. Boas, R.A. Dos Santos, A.C.Simões e Silva, *Vasc. Health Risk. Manag.,* **4**, 787 (2008).
20. H. Vural, T. Sabuncu, S.O. Arslan, N. Aksoy, *J. Pineal. Res.,* **31,** 193 (2001).
21. A. Armagan, E. Uz, H.R. Yilmaz, S. Soyupek, T. Oksay, N. Ozcelik, *Asian J. Androl.,* **8,** 595 (2006).
22. K. Yankov, D. Ilieva., *Tr. J. Sci.,* **13**, 420 (2015).
23. K.Yankov *Tr. J. Sci.,* **8**, 41 (2010).
24. R. Raikova, H. Aladjov, *J. Electromyogr. Kinesiol.*, **14**, 227 (2004).
25. K. Yankov, in: Proceedings of the International Conference on Information Technologies (InfoTech-2011), R. Romanski (eds.), 2011, p. 225.
26. K. Yankov, in: Proceedings of the International Conference on Information Technologies (InfoTech-2012), R. Romanski (eds.), 2012, p.114.
27. N. Bektaş, Y. Öztürk, *Turk. J. Med. Sci.*, **42**, 211 (2012).
28. G. Liu, F. Daneshgari, *Chin. Med. J. (Engl).*, **127,** 1357 (2014).
29. L.A. Szarka, M. Camilleri, *J. Diabetes Sci. Technol.,* **4**, 180 (2010).
30. V.J. Horváth, F. Izbéki, C. Lengyel, P. Kempler, T. Várkonyi, *Curr. Diab. Rep.*, **14**, 527 (2014).
31. L. He, Y. Sun, Y. Zhu, R. Ren, Y. Zhang, F. Wang, *Genet. Mol. Res.,* **13,** 7163 (2014).
32. S. Tobu, M. Noguchi, T. Hatada, K. Mori, M. Matsuo, H.Sakai, *Curr. Urol.*, **6**, 62 (2012).
33. K. Touw, S. Chakraborty, W. Zhang, A.G. Obukhov, J.D. Tune, S.J. Gunst, B.P. Herring, *Am. J. Physiol. Gastrointest. Liver. Physiol.,* **302**, 66 (2012).
34. Z. Jancsó, N. Bódi, B. Borsos, É. Fekete, E. Hermesz, *Int. J. Biochem. Cell Biol.,* **62**,125 (2015).
35. J.H. Han, I.H. Chang, S.C. Myung, M.Y. Lee, W.Y. Kim, S.Y. Lee, S.Y. Lee, S.W. Lee, K.D. Kim. *Korean J Physiol Pharmacol.,* **16**, 37 (2012).
36. I.M. Kvetnoy, I.E. Ingel, T.V. Kvetnaia, N.K. Malinovskaya, S.I. Rapoport, N.T. Raikhlin, A.V. Trofimov, V.V. Yuzhakov, *Neuro Endocrinol Lett.,* **23**, 121 (2002).
37. E. Peschke, *J. Pineal Res.*, **44**, 26 (2008).
38. E.C. Ebert, *Dis. Mon.,* **51**, 620 (2005).
39. H. Ariga, K. Imai, C. Chen, C. Mantyh, T.N. Pappas, T. Takahashi, *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **294**, 1807 (2008).
40. C. Yang, Z. Liu, K. Liu, P.Yang, *PLoS One.,* **9**, e85785 (2014)
41. D. Zhao, Y. Zhan, H. Zeng, M.P. Moyer, C.S. Mantzoros, C. Pothoulakis, *J. Cell. Biochem.,* **97**, 1317 (2006).
42. Z.Z. Liu, W.G. Wang, Q. Li, M. Tang, J. Li, W.T. Wu, Y.H. Wan, Z.G. Wang, S.S. Bao, J. Fei, *Cell Biosci.*, **5**, 12 (2015).

АНГИОТЕНЗИН II – ПРЕДИЗВИКАНА АКТИВНОСТ НА РЕЗЕРВОАРНИ ГЛАДКО-МУСКУЛНИ ОРГАНИ ОТ ТРЕТИРАНИ С ГРЕЛИН И МЕЛАТОНИН ДИАБЕТНИ ПЛЪХОВЕ

П. В. Хаджибожева[1*], Ц. К. Георгиев[1], Р. Е. Калфин[2], Г. С. Илиева[1], А. Н. Толекова[1]

[1]Катедра Физиология, патофизиология и фармакология, Медицински Факултет, Тракийски Университет, ул. „Армейска" 11, Стара Загора 6000 (България)
[2]Институт по Невробиология, Българска Академия на Науките, ул. „Акад. Георги Бончев" 23, София 1113 (България)

(Резюме)

Целта на изследването беше да се установи ефекта от краткосрочното приложение на грелин (ГРЛ) или мелатонин (МЛТ) върху ангиотензин II (Анг II)-провокирана активност на стомах, ректум и пикочен мехур от плъхове със стрептозотоцин (СТЗ)-предизвикан диабет. Половозрели плъхове, линия Wistar, бяха разделени в 4 групи: контрола; СТЗ-третирани: с единична доза СТЗ; МЛТ-третирани: единична доза СТЗ, последвано от приложение на МЛТ за 7 последователни дни; ГРЛ-третирани: единична доза СТЗ, последвано от приложение на ГРЛ за 7 последователни дни. Експериментът продължи 42 дни и в края му препарати от резервоарните органи бяха изработени и повлияни с Анг II. Анализът на силовите и кинетични параметри на получените гладко-мускулни съкращения бе осъществен със софтуер KORELIA.

СТЗ-предизвикания диабет засегна в различна степен Анг II-провокираната активност на резервоарните органи. При групата, третирана с МЛТ, се установи силен отговор на стомаха към Анг II (1.91±0.07 g) и слаба реакцията на препаратите от пикочен мехур (1.12±0.11 g), в сравнение с контролната група (съответно 1.14±0.13 g и 1.74±0.22 g). Приложението на ГРЛ почти напълно възстанови нормалните силови характеристики на съкращението на пикочния мехур и доведе до ускорено протичане на стомашните контракции. Отговорът към Анг II на ректалните препарати от третираните с ГРЛ или МЛТ животни, не беше подобрен.

Въпреки че бяха частични, се регистрираха благоприятни ефекти от краткосрочното приложение на МЛТ или ГРЛ при животните със СТЗ-индуциран диабет. Благотворният ефект върху Анг II-предизвиканата активност на стомах и пикочен мехур вероятно се дължи на антиоксидантните и про-кинетични въздействия на МЛТ и ГРЛ върху гладката мускулатура.