Bulgarian Chemical Communications, Special Issue E, (pp. 191 – 198) 2017

Effect of N-[N'-(2-chloroethyl)-N'-nitrosocarbamoyl-glycine amide of 2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl (SLCNUgly) on Angiotensin II-mediated smooth muscle activity of organs in pelvic cavity

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Received September 29, 2016; Revised February 21, 2017

Persistent hyperglycemia during diabetes mellitus impairs contractile responses of smooth muscles to pressor hormones like Angiotensin II (Ang II). The main etiological factor for this diabetic disturbance is the excessive formation of reactive oxygen radicals leading to oxidative stress and disrupted cell calcium signaling machinery. Therefore antioxidants have the potential to improve smooth muscle diabetic dysfunction.

The purpose of this study was to assess the effects of administration of SLCNUgly on the oxidative and glycemic status and on Ang II – induced motility of organs from the pelvic cavity of rats.

Mature female Wistar rats were divided into three groups: control group (intact animals); STZ-treated group (single injection of 60 mg/kg STZ); group, treated seven consecutive days after STZ injection with 10mg/kg SLCNUgly. In the end of experimental period, longitudinal strips from the urinary bladder, rectum and uterus were prepared and influenced by Ang II (1μmol). The obtained contraction curves were analyzed by calculation of force and time-parameters of the contractile process. The concentrations of ascorbate radicals, ROS production and lipid peroxidation (malondialdehyde) were evaluated in tissue homogenates from the liver, kidney and pancreas.

The seven-day administration of SLCNUgly improved significantly the glycemic status. It caused an additional reduction of Ang II-mediated response and greatly decreased the half relaxation phase of the myometrial response. Rectal preparations from SLCNUgly-treated diabetic rats responded to Ang II with reduced force parameters. The nitrosourea tends to normalize force and time-parameters of the urinary bladder. SLCNUgly has a small effect over amelioration of tissue oxidative damages.

Key words: Angiotensin II, SLCNUgly, smooth muscle contraction, oxidative stress, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) comprises a group of metabolic disorder characterized by varying or persistent hyperglycemia, due to decreased production of insulin or impaired utilization of glucose. DM is a major and increasingly significant worldwide health problem. World Health Organization predicts increase the incidence of diabetes to 5% for the period until 2030 [1]. The persistent hyperglycemia leads to long-term organ damages, thus affecting all the systems in the organism [2]. Most of the manifested symptoms of DM are related to smooth muscle dysfunction [3]. There are numbers of articles, which described the impaired urinary bladder activity [4], rectal incontinency [5] and uterine dysfunction [6]. The etiology of the impaired smooth muscle motility in DM is multifactorial [7], but the main factor for this diabetic disturbance is the excessive formation of reactive oxygen species (ROS) leading to oxidative stress and disrupted cell calcium signaling machinery [8]. Considering the role of oxidative stress in the development of structural and functional cell damage and the progression of DM, many scientific efforts are directed towards search and application of effective antioxidants [9]. Possible agent with therapeutic antioxidant potential for treatment of DM, is the newly synthetized nitrosourea N-[N'-(2-chloroethyl)-N'-nitrosocarbamoyl-glycine amide of 2, 2, 6, 6-tetramethyl- 4- aminopiperidine- 1- oxyl (SLCNUgly), spin-labeled analog of CCNU [10]. Previously reported *in vitro* physico-chemical properties determined higher alkylating activity, shorter half-life (29 min for SLCNUgly and 54 min for CCNU), and almost twice lower carbamoylating activity comparing to CCNU. *In vivo* SLCNUgly exhibited higher anti-leukaemic activity, anti-melanomic and immunomodulatory properties [11,12], and represent as a new class for tumor scintigraphy, radioprotectors which may have application as general antioxidant for *in vivo* radiotherapy and tumor localization [13].

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The purpose of this study was to assess the effects of administration of SLCNUgly on the oxidative and glycemic status and on Ang II – induced motility of organs from the pelvic cavity of rats.

EXPERIMENTAL

Animals

18 non-pregnant female Wistar rats, weighing 200-250 g were used. The animals were divided into the following groups: Group 1: controls; Group 2: diabetic animals; Group 3: diabetic animals, treated with SLCNUgly. DM was induced by a single intraperitoneal injection of Streptozotocin (STZ) 60 mg/kg BW. STZ was dissolved in cold 0.1M citrate buffer, pH 4.5. 72 hours after STZ administration, only animals with blood glucose levels higher than 16 mmol/l were considered to be diabetic and left in the experiment. The experiment lasted 8 days. SLCNUgly was administered in a dose of 10 mg/kg i.p. The application of SLCNUgly started in the next day, after STZ injection and lasted 7 consecutive days. The control group was injected with saline i.p. for 8 consecutive days.

Sample preparations and experimental protocols

In the end of experimental period, animals were anesthetized with Nembutal 50 mg/kg i.p. and preparations of the urinary bladder (UB), uterine horns (UH) and rectum (R) were made. The experimental protocol of the study was approved by the Institutional Animal Care and was in accordance with the national regulations and European Directive of 22.09.2010 (210/63/EU) concerning the protection of animals used for scientific and experimental purposes.

The preparation of the tissue samples and the recording of mechanical activity were conducted as it was previously described [14,15]. After the equilibration period, preparations were influenced by Ang II in a dose of 1 μmol (10^{-6} M).

Chemicals, drugs and equipment

Ang II, STZ and all reagents for preparation of Krebs solution and citrate buffer were purchased from Sigma-Aldrich Chemie GmbH, Germany. Blood glucose levels were measured by Medisign mm810 glucomer (Empecs Medical Device Co., Ltd., China).

Spin-labeled drug SLCNUgly was synthesized according to Zheleva et al. [10]. Dimethyl sulfoxide (DMSO), N-tert-butyl-alpha-phenylnitrone (PBN), 2-(4-carboxyphenyl)-4,4,5,5-tetra-methylimidazoline-1oxyl-3-oxide (Carboxy-PTIO.K) and PBS were purchased from Sigma Chemical Co, St. Louis, USA. All the other chemicals used in this study were with analytical grade.

Data analysis and statistical processing

The mechanical activity was transformed by a mechanical-force sensor, amplified, digitized and recorded using digital acquisition software ISOSYS-ADVANCED 1.0, produced by Experimetria Ltd., Hungary. The conversion of the data and primary data processing was performed with KORELIA-Processing software [16]. The recorded force-vs.-time curves permit determination of amplitudes of contraction and integrated force of contraction (represented by the area under the curve - AUC). The following time-parameters of smooth muscle contraction (SMC) were defined and calculated: half-contraction time (T_{hc}) - time interval between the beginning of SMC and half of the maximal force ($F_{max}/2$); contraction time (T_c) - time interval between the beginning of SMC and F_{max}; half-relaxation time (T_{hr}) - time interval between F_{max} and $F_{max}/2$; contraction plus half-relaxation time (T_{chr}) - time interval between the start of the SMC and $F_{max}/2$. The duration of the interval for analysis of tonic contraction was 5 minutes. Reported amplitudes, integrated force and time-parameters of uterine SMC were analyzed with KORELIA-Dynamics software [17].

For all Electron Paramagnetic Resonance measurements an X-band EMXmicro spectrometer (Bruker, Germany) equipped with standard Resonator was used. Spectral processing was performed using Bruker WIN-EPR and SimFonia software. The levels of the Asc., NO radicals and ROS products in the tissue/organ homogenates were calculated after double integration of the plots under the corresponding EPR spectra and expressed in arbitrary units. The level of ROS products was studied according to Shi et al. [18] with some modifications by Zheleva et al. [19]. Asc. radicals were studied by Buettner & Jurkiewicz [20], and •NO radicals according to methods of Yoshioka et al. [21] and Yokoyama et al. [22] with slight modifications.

The data obtained were processed by statistical program Statistica Version 6.1 (StaSoft, Inc., Tulsa, OK, USA) and presented as mean ± standard error. Statistical analysis was performed using one-way ANOVA and Student t-test to determine significant differences among data groups. A *P*-value less than or equal to 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Smooth muscle activity

Urinary Bladder

As is shown in Figure 1 and table 1, the registered force parameters of the UB preparations from group 2, were increased. Ang II-mediated response of the UB strips of group 3 was approaching to the controls. In regard to time-parameters, there was no statistical difference between the three groups.

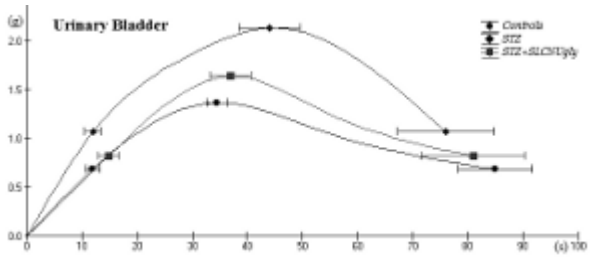

Fig. 1. Graphic visualization of SMC from rat urinary bladder after Ang II stimulation.

Table 1. Parameters of SMC from Urinary bladder of rats after Ang II stimulation.

	Ampl. (g)	AUC (gs)	T_{hc} (s)	T_c (s)	T_{hr} (s)	T_{chr} (s)
Con-trols	1.37± 0.19	120± 10	11.92± 1.36	34.5± 2	50.5± 4.6	84.9± 6.731
STZ	2.14± 0.41*	123.83± 229.62	10.79± 1.52	46.67± 5.5*	33.67± 6.93*	76± 8.87
SLCN Ugly	1.64± 0.33	94.83± 28.53	14.83± 1.94	37± 3.79	43.83± 6.69	80.8± 9.47

*P<0.05 vs. Controls.

Uterus

The amplitude and the AUC of the Ang II-mediated response of the UH from group 2 were significantly reduced compared to the controls (Fig. 2 and Table 2). Ang II-stimulated response of the UH from group 3 demonstrated a tendency to increase the force parameters of the SMC, but the statistical significance compared to the controls still existed. In regard to the time-parameters was observed greatly reduced T_{hr} and T_{chr} compared to the controls and group 2.

Rectum

In regard to rectal SMC Ang II-induced response of group 2 was with decreased amplitude and AUC compared to the controls (Fig. 3 and Table 3). Very interesting, the 7 consecutive day application of SLCNUgly caused an additional significant decrease in the amplitude and the AUC of the UH Ang II-provoked activity. With respect to the time-parameters there were no statistical significance differences between the three groups.

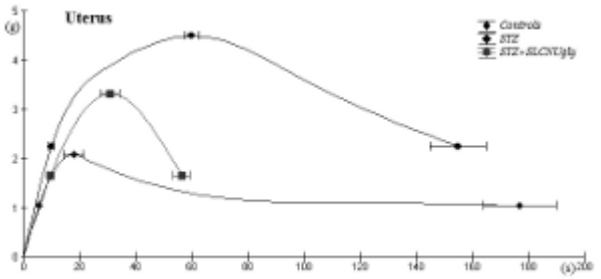

Fig. 2. Graphic visualization of SMC from rat uterus after Ang II stimulation.

Table 2. Parameters of SMC from Uterus of rats after Ang II stimulation.

	Ampl. (g)	AUC (gs)	T_{hc} (s)	T_c (s)	T_{hr} (s)	T_{chr} (s)
Con-trols	4.5± 0.35*	465± 31.4*	10± 1	60± 2.5*	95± 7.5*	155± 10
STZ	2.08± 0.28	219.25± 15.1	5.5± 0.22$^{\$}$	17.8± 3.6	159.4± 11	177± 13
SLCN Ugly	3.32± 0.28$^{\&}$	129.5± 10.75$^{\&}$	10.13± 1.22	32± 2.5$^{\&}$	24.5± 3$^{\&}$	56.5± 3$^{\#}$

*P<0.05 vs. STZ and SLCNUgly, $^{\#}P$<0.05 vs. Controls and STZ, $^{\&}P$<0.05 vs. STZ, $^{\$}P$<0.05 vs. Controls and SLCNUgly.

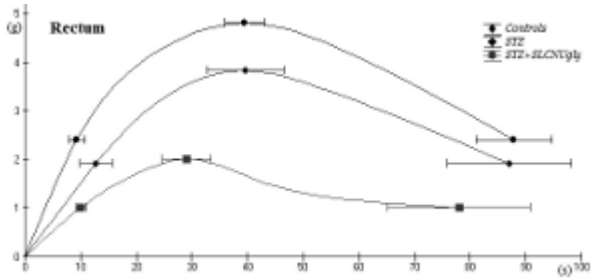

Fig. 3. Graphic visualization of SMC of rat rectum after Ang II stimulation.

Blood glucose levels

As it was expected, the animals treated only with a single STZ injection demonstrated a very high level of blood glucose. The rats injected with SLCNUgly 7 consecutive days presented significantly lower values compared to the STZ treated rats (Table 4).

Table 3. Parameters of SMC from Rectum of rats after Ang II stimulation.

	Ampl. (g)	AUC (gs)	T_{hc} (s)	T_c (s)	T_{hr} (s)	T_{chr} (s)
Controls	4.8± 0.44*	328.5± 75*	9.27± 1.52	39.43± 3.53	49.43± 3.3	88± 6.83
STZ	3.82± 0.42	192± 24	12.75± 2.86	39.58± 7.1	38.92± 5.47	87± 11.2
SLCNUgly	2.02± 0.29$^{\&}$	104.8± 14$^{\&}$	10.1± 1	29.2± 4.36$^{\#}$	49.1± 4.2	78.3± 12.8

*P<0.05 vs. STZ and SLCNUgly, $^{\#}P$<0.05 vs. Controls and STZ, $^{\&}P$<0.05 vs. STZ.

Table 4. Blood glucose level.

Blood Glucose	Controls	STZ	SLCNUgly
mM/L	6.8±0.3*	29±2.0	18.7±1.7$^{\&}$

*P<0.05 vs. STZ and SLCNUgly, $^{\&}P$<0.05 vs. STZ.

Ex vivo assay the levels of ROS products, Asc. and NO radicals, in tissue homogenates of rats by EPR spectroscopy.

ROS products

As can be seen in all three organs were not found statistically significant differences in levels of ROS products measured in group 1 and group 2 when compared with the control group (Fig. 4).

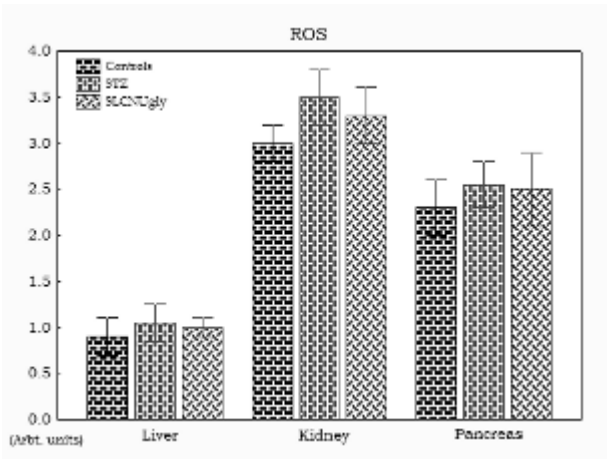

Fig. 4. Levels of ROS in tissues homogenates.

However, should be noted, that in the livers and kidneys of rats treated with STZ + SLCNUgly was found a reduction in the levels of ROS and bringing them to those of the control group. EPR settings were as follows: center field 3503 G; sweep width 10.0 G; microwave power 12.83 mW; receiver gain $1x10^6$; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 81.92 s; 5 scans per sample.

The nitric oxide levels.

In kidney homogenates were not statistically different levels of NO measured in STZ and STZ+SLCNUgly as compared to control group (Fig. 5). In the liver and pancreas homogenates of rats treated with STZ+SLCNUgly levels of NO radicals were statistically higher compared to the controls (Fig 5). The EPR settings were as follows: center field 3505 G; microwave power 6.42 mW; mod. amplitude 5 G; sweep width 75 G; gain 2.5x102; time constant 40.96 ms; sweep time 60.42 s; 1 scan per sample.

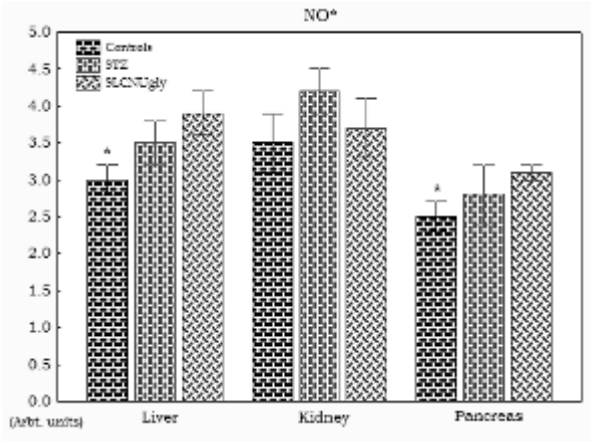

Fig. 5. Levels of NO* in tissues homogenates.
*P<0.05 vs. SLCNUgly

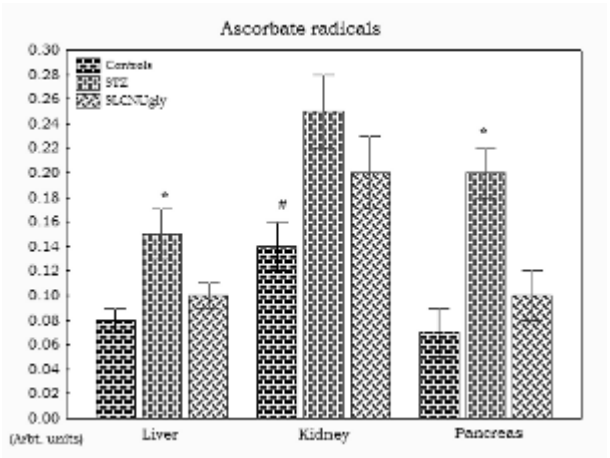

Fig. 6. Levels of Ascorbate radicals in tissues homogenates.
*P<0.05 vs. Controls and SLCNUgly, $^{\#}P$<0.05 vs. STZ and SLCNUgly.

Ascorbate radicals

As is seen from Fig. 6 the levels of ascorbate radicals in all three organ homogenates isolated from rats treated only with STZ were statistically significant higher than those of controls. It is

interesting to note that in the livers and pancreas of rats treated with STZ + SLCNUgly levels of Asc. were almost comparable to that of controls and statistically significant reduced comparing to that measured in the same organs of rats injected with STZ, only. Such a reduction is also found in the kidney homogenates, but the decrease was not statistically significant compared to STZ treated group. EPR settings were as follows: center field 3503 G; sweep width 10.0 G; microwave power 12.83 mW; receiver gain 1x106; mod. amplitude 5.00 G; time constant 327.68 ms; sweep time 81.92 s; 5 scans per sample.

Lipid peroxidation (MDA) in the tissue homogenates of rats measured spectrophotometrically:

As is seen the lowest levels of MDA were found in control group in all homogenates, but with no statistical difference compared to the other groups with exception of the pancreas of the rats, treated with STZ only (Table 5). It should be mentioned, that MDA levels measured in the homogenates of the group treated by STZ + SLCNUgly were closer to those of the controls when compared with the MDA levels of the STZ treated group.

Table 5. Levels of MDA in tissues homogenates.

MDA / microM	Liver	Kidneys	Pancreas
Controls	1.74±0.51	4.052±0.62	2.65±0.33
STZ	1.891±0.54	4.322±0.89	3.24±0.06*
SLCNUgly	1.86±0.65	4.137±0.73	2.89±0.72

*P<0.05 vs. Controls.

Smooth muscle activity

The Ang II-mediated response of the smooth muscle in the pelvic cavity was different in each organ.

In urinary bladder, there was no difference between the control group and the group with administration of STZ+SLCNUgly. In another hand, the group treated with STZ demonstrated hyper contractility of the bladder. In previously experiment lasting 42 days, we obtained no differences regarding the amplitude of SMC between diabetic and control group [23]. This different response in the present experiment is most likely due to the described from Nakahara et al. [24] negative feedback triggering by MaxiK channels. The authors found that there is hyper activity of the bladder due to higher sensitivity of the L-type Ca^{2+} channels, which in turn is compensated with MaxiK channels triggering feedback. Apparently, L-type Ca^{2+} channels high sensitivity is developing faster and 8 days were not enough to switch on K^+ channels feedback. The antioxidant properties of the SLCNUgly could explain the better response to Ang II of the UB in the group with SLCNUgly application.

The main feature of the uterus is predominantly expression of AT2 type receptors [25] in myometrium and the uterine artery [26]. Under this condition, the STZ application influenced negatively the Ang II- mediated uterine contractile activity, while the combination STZ+SLCNUgly demonstrated tendency to improve the amplitude of SMC and to reduce the relaxation. The neuropathy, myopathy [27] and reduction of calmodulin levels and Ca^{2+}-desensitization, caused by the hyperglycemia and ROS excess [28,29] are the main factors associated with these results. ROS concentration and pH determine developing of SMC [30,31]. This is why we could suppose that SLCNUgly as an agent with some antioxidant properties reduces the levels of oxidative stress in uterus, thus improving of the contractility. Also, it was very interesting that the application of SLCNUgly caused the significant decrease of relaxation phase. Activation of NO synthesis by endothelial cells [32] or alkylation of Ang II derivate [33] are possible reasons for development of this short relaxation. Moreover, the alkylation of AT1 receptors described by Dhanoa et al. [34] can disrupt the AT1 receptors signaling. Although for the developing of uterine SMC is necessary interaction between AT1 and AT2 receptors [35], leading role of AT2 receptors in this condition would lead to a fast relaxation.

Ang II-mediated response in rectum smooth muscle strips was the only one where the application of SLCNUgly caused a strong reduction of the amplitude of contraction. The other two groups – controls and STZ injected rats, demonstrated similar non-significant response. Nitrosoureas as alkylating agents are used in treatment of adenocarcinomas, especially in colorectal cancer [36]. SLCNUgly, as a new class nitrosourea, might have a high concentration in the rectum after 7 consecutive day application, which may cause an additional damage and reinforcement of STZ effects in the rectum.

In this study was observed a pronounced tendency in reducing the levels of blood glucose after 7 consecutive days of administration of SLCNUgly. Formerly, was demonstrated a statistically significant decrease in blood glucose

levels of healthy mice treated once with SLCNUgly [37]. The observed decrease in the level of blood glucose the same authors explained with the presence of glycine structure in SLCNUgly. In many studies was shown that per os intake of glycine causes increase in insulin concentration in the sera of healthy volunteers [38,39]. It is known that glycine participates in gluconeogenesis and any increase of its concentration in hepatocytes would cause disturbance in the control of blood glucose level.

Analysis of the results obtained for the levels of oxidative stress parameters namely, ROS products, Asc., NO and MDA reactive substances in both groups treated with STZ and combination of STZ + SLCNUgly showed that SLCNUgly behaves as an antioxidant comparing to STZ. Previously, was reported that due to presence of the stable nitroxyl radical structure (spin labeled) SLCNUgly could successfully scavenged ROS in particular superoxide radicals and to prevent formation of high toxic species like ·OH radicals [40]. It was documented that concentration of ascorbate free radical was a reliable, real-time and quantitative marker of free radical generation could be used as an indicator of oxidative stress *in vitro* and *in vivo* [20,41]. Based on both: 1) Levels of ascorbate radicals registered in the three organ homogenates of the group treated with STZ + SLCNUgly were close to those of the control group and 2) were considerably lower than those registered in the group treated only with STZ, might be concluded that obviously SLCNUgly induced to a lesser extent oxidative stress than the other nitrosourea. We believe that this drug has double effect over blood sugar regulation. On the one hand, we can speculate that SLCNUgly has direct effect on insulin release by closing of ATP-sensitive K-channels in the beta-cell plasma membrane similar to the anti-diabetic drug sulfonylurea [42]. On the other hand, both SLCNUgly and STZ are nitrosoureas. We believe that there might be a competition between them for the GLUT 2 transporter. In this way, the additional administration of SLCNUgly leads to a prevention of the toxic effects of STZ.

CONCLUSION

STZ induced hyperglycemia and oxidative stress produced different changes in Ang II-provoked motor activity of organs in pelvic cavity: from hypo reactivity - uterus, no changes - rectum to hyper reactivity - UB. The effect of the antioxidant protection depends on different functional characteristics of pelvic organs and interplay between Ang II receptors and SLCNUgly.

The seven-day administration of SLCNUgly improved significantly glycemic status of the rats, but obviously the duration of the treatment with SLCNUgly or/and the dose was not enough for the complete amelioration of tissue oxidative damages and to restore oxidative balance. The beneficial effect of SLCNUgly allows us to a future development of our research in this direction.

Acknowledgements: *This study was supported by Grant DDVU-02-24/2010 from National fund, Sofia, Bulgaria and Grant 25/2014 from Trakia University, Stara Zagora, Bulgaria.*

REFERENCES

1. A. Amos, D. Mccarty, P. Zimmet, *Diab. Med.*, **14**, 1 (1997).
2. A. M. Abu El-Asrar, *Middle East Afr. J. Ophthalmol*., **20**, 273 (2013).
3. J. Stamler, O. Vaccaro, *J. D.* Neaton, *Diabetes Care*, **16**, 434 (1993).
4. C. Frimodt-Moller, *Ann. Int. Med*., **92**, 318 (1980).
5. L. R. Schiller, C. A. Santa Ana, A. C. Schmulen, R. S. Hendler, W. V.. Harford, J. S. Fordtran, *N. Engl. J. Med*., **307**, 1666 (1982).
6. G. Falkay, G. Spiegl, D. Csonka, I. Zupkó, *Neurochem. Int*., **51**, 306 (2007).
7. M. J. Chang, J. H. Xiao, Y. Wang, Y. L. Yan, J. Yang, J. L. Wang, *PLoS One*., **7**, 50291 (2012).
8. T. J. Lyons, *Diabet Med.*, **8**, 411 (1991).
9. A. Chatzigeorgiou, A. Halapas, K. Kalafatakis, E. Kamper, *In Vivo*, **23**, 245 (2009).
10. A. Zheleva, Z. Raikov, M. Ilarionova and D. Todorov, *Pharmazie*, **50**, 25 (1995).
11. M. V. Ilarionova, A. M. Zheleva, Z. D. Raikov, D. K. Todorov, A. P. Dudov, *J. Chemotherapy*, **5**, 786 (1993).
12. A. Zheleva, S. Stanilova, Z. Dobreva, Z. Zhelev, *Int. J. Pharmaceutics*, **222**, 237 (2001).
13. Y. Karamalakova, K. Chuttani, R. Sharma, A. Zheleva, V. Gadjeva, A. Mishra, *Biotechnol. & Biotechnol. Equip.*, **28**, 1172 (2014).
14. A. Tolekova, P. Hadzhibozheva, R. Iliev, Ts. Georgiev, K. Trifonova, R. Sandeva, R. Kalfin, G. Ilieva, *Reg. Pep.*, **162**, 79 (2010).
15. Ts. K. Georgiev, P. V. Hadzhibozheva, A. N. Tolekova, *Scripta Sci. Med.*, **44**, 93 (2012).
16. K. Yankov, *Trakia J. Sci.*, **8**, 41 (2010).
17. K. Yankov, in: Proceedings of the International Conference on Information Technologies (InfoTech-2012), R. Romanski (edr), 2012, p.114.
18. H. H. Shi, Y. X. Sui, X. R. Wang, Luo Yand, L. L. Jia, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **140**, 115 (2005).
19. A. Zheleva, Y. Karamalakova, G. Nikolova, R. Kumar, R. Sharma, V. Gadjeva, *Biotechnol. & Biotechnol. Equipment*, **26**, 146 (2011).
20. G. R. Buettner and B. A. Jurkiewicz, *Free Radic. Bio. Med.*, **14**, 49 (1993).

21. T. Yoshioka, N. Iwamoto, and K. Ito, *J. Am. Soc. Nephrol.*, **7**, 961 (1996).
22. K. Yokoyama, K. Hashiba, H. Wakabayashi, K. Hashimoto, K. Satoh, T. Kurihara, N. Motohashi and H. Sakagami, *Anticancer Res.,* **24**, 3917 (2004).
23. P. Hadzhibozheva, PhD Thesis, INB, BAS, Sofia 2015.
24. T. Nakahara, A. Mitani, Y. Kubota, T. Maruko, K. Sakamoto, Y. Tanaka, K. Koike, K. Shigenobu, K. Ishii, *J. Smooth Muscle Res*., **40**, 97 (2004).
25. T. Matsumoto, N. Sagawa, M. Mukoyama, I. Tanaka, H. Itoh, M. Goto, H. Itoh, M. Horiuchi, V. J. Dzau, T. Mori, K. Nakao, *J. Clin. Endocr. Metab.*, **81**, 4366 (1996).
26. R. E. Hannan, T. A. Gaspari, E. A. Davis, R. E. Widdop, *Brit. J. Pharmacol.*, **141**, 1024 (2004).
27. L. A. Szarka, M. Camilleri, *J Diabetes Sci Technol*., **4**, 180 (2010).
28. Y. Ozturk, S. Aydin, A. T. Ozcelikay, V. M. Altan, N. Yaldizoglu-Ari, *Eur. J Pharmacol*., **31**, 59 (1997).
29. H. Suzuki, I. Usui, I. Kato, T. Oya, Y. Kanatani, Y. Yamazaki, S. Fujisaka, S. Senda, Y. Ishii, M. Urakaze, A. Mahmood, S. Takasawa, H. Okamoto, M. Kobayashi, K. Tobe, M. Sasahara, *Diabetologia*, **54**, 2953 (2011).
30. I. Appiah, S. Milovanovic, R. Radojicic, *Brit. J. Pharmacol*, **158**, 1932 (2009).
31. R. C. Heaton, M. J. Taqqart, S. Wray, *Pflugers Arch*., **422**, 24 (1992).
32. V. M. Rudichenko, W. H. Beierwaltes, *J. Vasc. Res*., **32**, 100 (1995).
33. K. Hsieh, G. R. Marshall, *J. Med. Chem*., **24**, 1304 (1981).
34. D. S. Dhanoa, S. W. Bagley, R. S. L. Chang, V. J. Lotti, T. B. Chen, S. D. Kivlighn, G. J. Zingaro, P. K. S. Siegl, A. A. Patchett, W. J. Greenlee, *J. Med. Chem.,* **36**, 4230 (1993).
35. Ts. K. Georgiev, A. N. Tolekova, R. E. Kalfin, P. V. Hadzhibozheva, *Physiol. Res.*, in press
36. M. E. Katz, J. H. Glick, *Cancer Clin. Trials*., **2**, 297 (1979).
37. A. Zheleva, A. Tolekova, P. Goicheva, *Trakia J. Sci.*, **6,** 49 (2008).
38. M. Gannon, J. A. Nuttall, F. Q, Nuttall, *Am. J. Clin. Nutr.*, **76**, 1302 (2002).
39. M. Gonzalez-Ortiz, R. Medina-Santillan, E. Martinez-Abundis, C. R. von Drateln, *Horm. Metab. Res.*, **33**, 358 (2001).
40. A. M. Zheleva, V. G. Gadjeva, *Int. J. Pharmaceutics*, **212**, 257 (2001).
41. http://slideplayer.com/slide/6626563/.
42. F. M. Ashcroft, *Horm. Metab. Res.,* **28**, 456 (1996).

ЕФЕКТ НА N-[N'-(2-ХЛОРОЕТИЛ)-N'-НИТРОЗОКАРБАМОИЛ-ГЛИЦИН АМИД НА 2,2,6,6-ТЕТРАМЕТИЛ-4-АМИНОПИПЕРИДИН-1-ОКСИЛ (SLCNUGLY) ВЪРХУ АНГИОТЕНЗИН 2-МЕДИИРАНАТА ГЛАДКОМУСКУЛНА АКТИВНОСТ НА ОРГАНИ В ТАЗОВАТА КУХИНА

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Постъпила на 29 септември, 2016 г.; Коригирана на 21февруари, 2017 г.

(Резюме)

Хипергликемията, съпътстваща захарния диабет нарушава отговора на гладките мускули към хормони с контрактилна активност като Ангиотензин 2 (Анг 2). Основният етиологичен фактор за това смущение е прекомерното образуване на кислородни радикали, което води до оксидативен стрес и нарушение на калциевата сигнализация в клетките. Ето защо веществата с антиоксидантна активност имат потенциал за подобряване на гладкомускулната диабетна дисфункция.

Целта на това проучване е да се оцени въздействието на SLCNUgly, върху оксидативния и гликемичния статус на диабетни плъхове, както и Анг 2 - индуцираната съкратителна активност на органи от тазовата кухина.

Женски полово зрели плъхове, линия Wistar, бяха разделени в три групи: контролна група (здрави животни); Стрептозотоцин (СТЗ) третирана група (единична инжекция от 60 мг/кг); група, третирана седем последователни дни, след СТЗ инжектирането с 10 мг/кг SLCNUgly. В края на експерименталния период, бяха изготвени надлъжни гладкомускулни ивици от пикочен мехур, ректум и матка, на които бе въздействано с Анг 2 (1микромол). Получените контрактилни криви бяха анализирани чрез изчисляване на силови и времеви характеристики на процеса. В тъканни хомогенати от черен дроб, бъбреци и панкреас, бяха изчислени концентрациите на аскорбатни радикали, продукцията на кислородни радикали и липидната пероксидация (малондиалдехид).

Седемдневното приложение на SLCNUgly подобри значително гликемичния статус на плъхчетата. Също така нитрозуреята причини допълнително намаление на Анг 2-медиирания контрактилен отговор и значително скъси фазата на полурелаксация на миометралната контракция. Препаратите от ректум изготвени от SLCNUgly-третирани диабетни плъхове, отговориха на Анг 2 стимулацията с намаление във силовите параметри на контракцията. Приложението на нитрозоуреята показа тенденция за нормализиране на силовите и времевите характеристики на контрактилния процес при препаратите от пикочен мехур. SLCNUgly имаше слаб ефект по отношение на подобряване тъканните увреди вследствие от оксидативния стрес.