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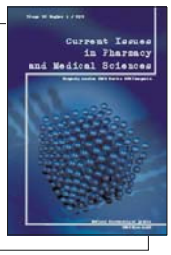

The changes of NO-synthases activity, nitrogen oxide content and lipoperoxidation processes in stomach and colon under conditions of streptozocin-induced hyperglycaemia

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ABSTRACT

In rats with streptozocin-induced hyperglycaemia the changes of nos activity, nitrite anion content and lipoperoxidation processes in mucous and muscular layers of the stomach and large intestine were studied. It has been showed that hyperglycaemia induces the decrease of cnos activity in mucous layer, while its activity in muscular layer of the stomach and large intestine did not change significantly and łnos activity increased in muscular layer of the stomach and large intestine. Nitrite anion content did not change significantly. Hyperglycaemia caused an increase of the lipoperoxidation processes in mucous and muscular layers of the large intestine; simultaneously sod activity in mucous and muscular layer increased.

Keywords: NO-synthase, nitrogen oxide, lipoperoxidation, stomach, hyperglycaemia

INTRODUCTION

In health the constitutive calcium-dependent forms of NO-synthases (NOS) – endothelial (eNOS) and neuronal (nNOS), are being expressed in mucous and muscular layers of the digestive organs [6, 7]. Constitutive forms of NOS provide biosynthesis of insufficient amount of NO, which regulates the processes of secretion, motor function, absorption, blood flow, participates in maintenance of the integrity and function of the mucous barrier, the processes of intercellular integration, transfer of information in non-adrenergic and non-cholinergic neurons [6,13].

nNOS was identified in 29% of mienteral neurons. Ninety percent of neurons (of 29%), in which NOS was detected, were the inhibitory motor neurons [8]. NO, synthesised by nNOS, plays the role of neurotransmitter and participates in the development of entheropathies, including Crohn's disease, Hakas disease, colitis ulcerosa, diabetes mellitus [1].

The inducible NO-synthase (iNOS) is being activated by different factors, among which bacterial lippopolysacharides, mitogenic stimuli, proinflammatory cytokins. Expression of iNOS n leads to acute increase (10 times and more) of NO production, from which nitrooxyl (NO^-) and peroxynitrite ($ÎNOO^-$) are being formed [3].

Under coditions of hyperglycaemia the digestive glands secretion, motor-evacuating function of the stomach and large intestine decrease and lipoperoxidation processes increase.

The aim of the study was to evaluate the changes of NOS activity, nitrite-anion content and lipoperoxidation processes in mucous and muscular layer of the stomach and large intestine under conditions of streptozocin-induced hyperglycaemia.

MATERIAL AND METHODS

The Ethical Committee of Lviv National Medical University approved the structure of this study and animal experimental procedures. The experiment was performed on 20 white male albino rats weighing 200-250g. They were divided into 2 groups: first – intact animals were used as controls, second – animals were provided with intraperitoneal injection of streptozocin in dose of 60 mg per kg of body weight. After 14 days when the diabetes mellitus was developed (fasting blood glucose level rose to 15-16 mmol/l) rats were sacrificed by decapitation under general anesthesia. Measurement was performed in homogenates of mucosal and muscular membranes of stomach and large intestine.

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The content of nitrite anion in homogenate was determined as nitrites by the method of Green L., David A. [4]. The absorbance was read in a Stat fax at 550 nm. Nitrite anion concentration was expressed in μmol/g. NOS (total NOS, iNOS, eNOS) activity was measured by the method described in detail [10]. NOS activity was expressed in nmol NADPH/min•g of protein.

Lipid peroxidation level was expressed as malonic dyadehyde (MDA) concentration in homogenates. It was measured according to the procedure of Timirbulatow et al. [11]. Catalase activity was determined by measuring of the decrease in hydrogen peroxide concentration at 410 nm by the Korolyuk method [5]. Activity of superoxide dismutase (SOD) was determined by the reaction of reduction of nitrotetrazoliume blue to nitroformazan [2].

Experimental results were analyzed by ANOVA and t-tests for multiple comparisons between groups. P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In control animals both in mucous layer of the stomach and large intestine, cNOS activity was dominating and approximately on the same level, łNOS activity was not significant (Table 1). Activity of ńNOS in muscular layer of the stomach and large intestine was smaller than in mucous layers.

Table 1. NOS activity and nitrite anion content in stomach and large intestine under conditions of streptozocin-induced heperglycaemia

	Nitrite anion (μmol/g)	NOS (nmol/min·g of protein)	iNOS (nmol/min·g of protein)	ńNOS (nmol/min·g of protein)
Mucous layer of the stomach				
Control	16.1±1.6	0.647±0.13	0.180±0.06	0.460±0.03
Hyperglycaemia	20.9±1.8	0.472±0.05	0.337±0.09*	0.135±0.08
Muscular layer of the stomach				
Control	15.1±1.3	0.496±0.17	0.183±0.06	0.335±0.12
Hyperglycaemia	19.8±1.2	0.569±0.08	0.326±0.08*	0.248±0.1
Mucous layer of the large intestine				
Control	17.4±2.1	0.699±0.15	0.236±0.08	0.498±0.13
Hyperglycaemia	20±1.5	0.445±0.07*	0.268±0.05	0.177±0.07*
Muscular layer of the large intestine				
Control	16.9±1.7	0.442±0.19	0.188±0.05	0.267±0.07
Hyperglycaemia	21±1.5	0.816±0.3*	0.466±0.2*	0.350±0.15

* statistically significant differences compared to control; p.05

The course of lipoperoxidation processes, SOD and cathalase activity in control animals were higher in mucous layers of the stomach and large intestine compared to the indices in muscular layer (Table 2).

Under conditions of hyperglycaemia ńNOS activity in mucous layer of the stomach decreased by 70% ($p<0.05$), in mucous layer of the large intestine — by 68% ($p<0.05$), while ńNOS activity in muscular layers did not change significantly. In mucous layer of the stomach łNOS activitó under conditions of hyperglycaemia increased by 87% ($p<0.05$), in mucous layer of the large intestine – by 2.5 times ($p<0.05$). Nitrite anion content did not change significantly.

Table 2. Lipoperoxidation processes, SOD and catalase activity in stomach and large intestine under conditions of streptozocin-induced hyperglycaemia

	MDA μmol/g	SOD μmol/minmg of protein	Cathalase μmol H_2O_2/minmg
Mucous layer of the stomach			
Control	222.1±8.7	22.3±1.4	0.3±0.04
Hyperglycaemia	247±13.3	28.4±6.2	0.4±0.08
Muscular layer of the stomach			
Control	135±13.4	18.5±1.2	0.12±0.01
Hyperglycaemia	145±14.0	23.6±3.5	0.14±0.01
Mucous layer of the large intestine			
Control	247±10.2	17.2±1.2	0.33±0.04
Hyperglycaemia	312±10.0*	22.3±1.4*	0.29±0.01
Muscular layer of the large intestine			
Control	97.2±15.5	16.6±1.4	0.15±0.01
Hyperglycaemia	148±7.0*	24.0±1.6*	0.16±0.01

* statistically significant differences compared to control; p.05

Hyperglycaemia induced an increase of lipoperoxidation processes – the content of TBA products the most significantly increased in mucous layer (by 26%, $p<0.05$) and muscular layer of the large intestine (by 52%,$p<0.05$); simultaneously SOD activity increased – by 30% in mucous layer and by 44% in muscular layer of the large intestine.

Under conditions of hyperglycaemia in rats the rate and duration of muscular contractions of the stomach decreased as well as the speed of spread of the contractions and the pressure of gastro-esophageal sphincter, the time of passage through the large intestine increased, all of which cause the stasis of chymus in the large intestine [9, 14, 15]. NOS/NO directly participate in these processes. It should also be mentioned that the acute relaxation of the smooth muscles of the stomach and large intestine takes place due to the increasing activity of iNOS. At the same time nitrite anion content did not change significantly, what can be mediated by its interaction with superoxide radical and peroxynitrite production.

NO regulates the functional status of the smooth muscles of the digestive system by several mechanisms. On the one hand NO, synthesised in NO-ergic neurons of the mienteral plexus acts as inhibitory mediator and on the other hand it might activate the instant guanilatciclase, causing the production of cGMF in smooth musles and their relaxation. Nitrogen oxide also may cause the muscular relaxation via hyperpolarisation and cGMF-independent way [12].

It is assumed that motoric impairment under conditions of diabetes mellitus is associated with nitritergic neuropathy due to the decrease of nNOS activity, which might be associated with the degenerative changes in nitritergic neurons or with impairment of nNOS expression. On the other hand, increase of iNOS activity significantly decreases the motor proprties of the large intestine [8].

CONCLUSION

Streptozocin-induced hyperglycaemia causes the decrease of ńNOS activity in mucous layers of the stomach and large intestine and increase of iNOS activity, lipoperoxidation processes and SOD activity, all of which contribute to the imparment of the motor-avacuating function of the stomach and large intestine.

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