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**A CONVENIENT METHOD TO SYNTHESIZE 3-CHLOROIMIDAZO[1,5-*a*]QUINOXALINES**

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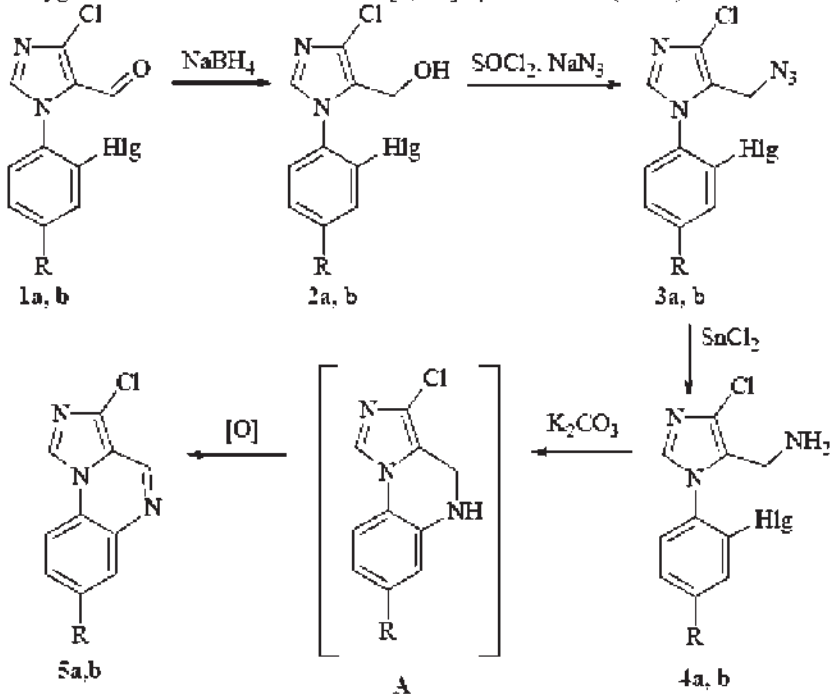
Imidazo[1,5-*a*]quinoxaline system is a promising background to design new biologically important objects. Annelation of quinoxaline cycle to imidazole is the most popular approach to synthesize of imidazo[1,5-*a*]quinoxaline derivatives. Cyclization of 5-unsubstituted 1-(2-aminoaryl)imidazole with the one-centered electrophylic reagents or intramolecular cyclocondensation of their 5-oxoderivatives are usually used for this synthesis.

We have found that the pyrazine cycle of imidazo[1,5-a]quinoxaline can be formed by the non-catalytic intramolecular N-arylation of 1-(2-halogenaryl)-5-aminomethylimidazoles. This synthesis was based on 1-(2-halogenaryl)-5-formyl-4-chloroimidazoles (1 a,b). Then they were transformed into intermediate 5-hydroxymethyl (2 a,b) and 5-azydomethyl derivatives (3 a, b) using some simple methods and then these compounds were transformed into 5-aminomethyl-1-(2-halogenaryl)-4-chlorimidazoles (4 a, b). Then the latter compounds were boiled in DMFA during 20 hours with $K_2CO_3$ that resulted in formation of 4,5-dihydroimidazoquinoxalines A, followed by their oxidation by the air oxygen into novel 3-chloroimidazo [1,5-a]xquinoxalines (5 a, b).




1-4, R = H, Hlg = Cl (a); R = Me, Hlg = Br (b); **5**, R = H (a), Me (b)

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**EFFECT OF TWO WEEKS DAILY MELATONIN INJECTION ON CHANGES IN THE CONTENT OF REDUCED GLUTATHIONE IN MUSCLES OF RATS WITH ALLOXAN DIABETES**

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Diabetes refers to the most common endocrine diseases. In diabetes, in addition to disorders of the metabolism of carbohydrates, lipids and proteins, there is also the disruption of antioxidant defense, including glutathione system.

Metabolic disorders in diabetes occur especially in insulin-dependent organs. Skeletal muscles belong to insulin-dependent tissues.

In recent years, hypoglycemic and antioxidant effect of exogenous melatonin has been established.

The aim of this investigation was to determine the effect of two weeks daily melatonin injection on the content of reduced glutathione in skeletal muscles of rats.

The experiments were carried out on 18 mature male albino rats with the body weight 0,18 – 0,20 kg. Alloxan diabetes was induced via injecting the rats with 5% solution of alloxan monohydrate intraperitoneally in a dose of 170 mg/kg of body weight. The animals were divided into three subgroups: 1) control group; 2) diabetic rats; 3) diabetic animals which were introduced the melatonin intraperitoneally in a dose of 10 mg/kg of body weight at 8 a.m. daily during 14 days starting with a 5-th 24 hour period after the injection of alloxan. Tissues of skeletal muscles were taken immediately after the decapitation of animals and used to prepare 10% homogenates on 6% sulfosalicylic acid. Determination of reduced glutathione (RG) was conducted by a titration method by I.V. Meschyshen. Statistical analysis of results was conducted by Student's test. The results of the study were statistically processed by means of

Student t-criterion after preliminary checking of value distribution in samples according to Shapiro-Wilk criterion. To enhance the reliability of the conclusions non-parametric Mann-Whitney criterion of comparison was parallel used. Sufficient divergence reliability level was considered as $r \leq 0,05$.

According to the results, the content of RG in the skeletal muscles of alloxan diabetic rats was 34% lower than in the muscles of control group animals. In group of alloxan diabetic rats the administration of melatonin intraperitoneally in dose of 10 mg/kg at 8 a.m. daily during 14 days the RG content did not differ from the group of intact rats. Decrease of RG content in skeletal muscles of alloxan diabetic rats indicated the reduction of glutathione branch function of antioxidant protection in conditions of obvious insulin deficiency. There are some possible reasons of RG reduction during diabetes mellitus – the increased use of RG by glutathione-dependent enzymes, disturbances in RG synthesis or reconstruction from its oxidized form due to NADPH deficiency (glucose-6-phosphate dehydrogenase enzyme – main source of NADPH – activated by insulin).

It is well known that pinealectomy leads to decrease of melatonin synthesis and secretion, which causes insulin resistance and reduces the gene expression of glucose transporter GLUT 4 in muscles.

According to the results of the study, administration of melatonin intraperitoneally in dose of 10 mg/kg at 8 a.m. daily during 14 days to alloxan diabetic rats prevents from exhaustion of RG reserves in rat muscles. Positive influence of melatonin is presumably mediated by its direct antioxidant action and activation of antioxidant enzymes.

Thus, the content of reduced glutathione in skeletal muscles of alloxan diabetic rats is decreased. The introduction of melatonin intraperitoneally in a dose of 10 mg/kg at 8 a. m. daily during 14 days to alloxan diabetic rats can assist to normalize the content of reduced glutathione in skeletal muscles.

**Lenga E.L.**

## CHANGES OF TOTAL ANTIOXIDANT ACTIVITY OF BLOOD SERUM IN RATS UNDER DIABETES AND ADMINISTRATION OF MELATONIN

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Experimental diabetes mellitus (DM) in rats is not only a model of carbohydrate metabolism disorders, but also is an example of free radical pathology. The increased formation of reactive oxygen species (ROS) for this disease is caused by activation propiolic way of glucose oxidation and, as a consequence - the depletion $NADFN_2$ content which reduces the content of reduced glutathione. In addition, increased content of glucuronidation final products, that promotes the formation of ROS such as superoxide anion, hydroperoxyl radicals, hydroxyl radicals, peroxynitrite and nitrogen dioxides. All these factors determine not only the development of oxidative stress, but the cause of diabetes complications such as angiopathy, which is the cause of retino-, nephro- and polyneuropathy.

The aim of this work was to investigate changes of total antioxidant activity in blood serum of rats with experimental diabetes mellitus (DM) in combination with nephropathy and their correction by melatonin (MT).

The research was carried out on non-linear white male rats weighing 160±20 g. The experiment was performed according to the requirements of the "European Convention for the Protection of vertebrate animals used for experimental and scientific purposes" (ETS №123) (Strasbourg, 18 March 1986). Experimental diabetes was caused by intraperitoneal administration of 5% solution alloxan monohydrate 150 mg / kg. Nephropathy was induced after five days by injection of 50% aqueous glycerol solution (10 ml / kg) to both thigh muscles. Melatonin was administered intragastrically 10 mg / kg for the following 14 days. Euthanasia was performed by decapitation of rats under light ether anesthesia on the 7th and 14th days from the beginning of melatonin administration.

The total antioxidant activity of blood serum (TAOS) was expressed as a percentage inhibition of spontaneous peroxidation of endogenous lipids of the brain (the content of malonic dyaldehide). The results were statistically processed by means of parametric Student's test with statistically significant difference $p<0.05$.

The studies have shown that TAOS in animals with diabetes was 41% significantly lower as compared to the control group of animals on the 7$^{th}$ day of the experiment. The combination of diabetes with nephropathy caused TAOS decrease by 55% as compared to the animals of the control group and 7% than in animals with diabetes. Administration of melatonin found no positive effect both in animals with diabetes and in animals with diabetes in combination with nephropathy.

On the 14$^{th}$ day of the experiment in animals with diabetes TAOS was found to be 44% lower as compared to the control group of animals. Administration of melatonin for 14 days caused 36% increase of the parameter as compared to the control animals and 34% higher than in animals of the same group on the 7$^{th}$ days of administration. In animals with a combination of diabetes and nephropathy TAOS was found to be 63% decrease as compared to the control animals by 32% than in animals with diabetes and 28% lower than in the corresponding group of animals on the 7$^{th}$ day of the experiment. Administration of melatonin promoted half increase of TAOS as compared to untreated animals and 63% higher than in animals on the 7$^{th}$ day of the experiment.

Thus, according to the results of the research, alloxan-induced diabetes, especially in combination with nephropathy causes a significant decrease of total antioxidant activity in serum of rats due to excessive activation of ROS formation. Administration of melatonin was more effective to restore total antioxidant serum resistance on the 14$^{th}$ day of the experiment, indicating its usage as a direct scavenger of free radicals in the early stages of oxidative stress. A prolonged administration of the drug had a positive effect not only due to the direct disposal of ROS, but also as a result of its protective effect on antioxidant enzymes and processes of their synthesis.