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UDC 615.252.349.7:616.37:616.379-008.64

INVESTIGATION OF THE ROLE OF I_1- AND I_2-IMIDAZOLINE RECEPTORS IN THE MECHANISM OF THE HYPOGLYCEMIC ACTION OF N,N'-(ETHANE-1,2-DYYIL)BIS(QUINOLINE-2-CARBOXAMIDE)

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Key words: N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide); hypoglycemic effect; imidazoline receptors; diabetes mellitus; 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride (BU 224); efaroxan; alloxan

The results of the investigation of the hypoglycemic action mechanism of N,N'-(ethane-1,2-dyyil)bis (quinoline-2-carboxamide) are presented. The substance was administered intragastrically in the dose of 11.64 mg/kg on the model of alloxan-induced diabetes mellitus in rats. It should be noted that the test compound in terms of the theory of pharmacophore can be considered as a dimer BU 224, but it is not its dimer in terms of organic chemistry. The information about the influence of the test compound on carbohydrate metabolism is limited. There are data that this compound has antitumor properties in vitro due to enhanced apoptosis and activation of caspase-3. Proceeding from the chemical structure, the 2-substituted quinoline fragment is present in the structure of the known antagonist of imidazoline receptors I_2 such as 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride, so it can be assumed that N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) has the ability to interfere with the mechanisms of the carbohydrate metabolism regulation. These results indicate that N,N'-(ethane-1,2-dyyil)bis(quinolin-2-carboxamide) has an expressed hypoglycemic effect in alloxan-induced diabetes mellitus reducing the blood glucose level by 71.50%. It is an agonist of imidazoline receptors of both types, and it has been proven by blockade with selective antagonists such as efaroxan and 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride. On the background of I_1-imidazoline receptors blocker efaroxan the glycemia decrease was 45.66% ($p<0.05$), and on the background of I2-imidazoline receptors blocker 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride it was 54.01% ($p<0.05$).

Diabetes mellitus (DM) type 2 is a chronic disease caused by decrease in sensitivity of the human tissues to insulin [1, 14]. The frequency of diabetes on average ranges from 1.5% to 3%; it is constantly increasing in the developed countries up to 5-7%, in Ukraine the value is twice lower – about 2.9%. There are about 200 million of diabetics in the world; almost 90% of them suffer from type 2 diabetes [5, 15].

The urgency of DM problem is caused by a significant prevalence of the disease, severity of complications, disability and early mortality. Microangiopathies and neuropathies are the basis of diabetic complications. The patients with diabetes have a significant risk of atherosclerosis and coronary heart disease. More than 40% of amputations of lower limbs are a consequence of the diabetic foot syndrome. Diabetes is also the most common cause of blindness in humans [12, 17]. All mentioned above stipulates the necessity of development and research of new effective drugs with the hypoglycemic effect allowing to prevent development of the severe course of DM.

The mechanism of action of different antidiabetic drugs consists of a several links. There are data on the role of imidazoline receptors in implementation of the hypoglycemic effect, in particular for biguanides derivatives such as metformin [7].

Imidazoline receptors are the independent type of receptors represented by two subtypes: 1_1 and 1_2. Subtypes are distinguished according to the specific ligands that bind with them. Imidazoline receptors mediate the following effects: increase of glucose-dependent insulin release and transport of glucose into the cells and, as a result, decrease of hyperglycemia, improvement of the energy supply of tissues by increasing aerobic oxidation of glucose and increase of glycogen synthesis, reduction of lactate production, increase of sensitivity of brain tissues to glucose, intensification of lipolysis, increase of sensitivity to blood pressure decreasing, as well as to hypoxia/hypercapnia of carotic glomeruli [10, 11].

Our attention has been attracted by a new compound that has a hypoglycemic effect on the model of alloxan diabetes in different routes of administration [13]. The research of the possible role of I_1- and I_2-imidazoline receptors in the mechanism of the action of N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide), which can act as a ligand to I_2-imidazoline receptors and contains two relevant pharmacophore fragments (Fig. 1), is of special interest.

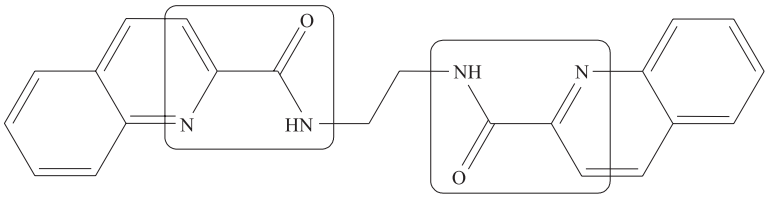

Fig. 1. N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) with the pharmacophore fragments selected – ligands of I_2-imidazoline receptors.

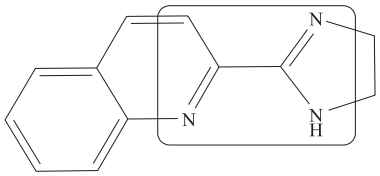

Fig. 2. 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride (BU 224) with the pharmacophore fragments selected – ligands of I_2-imidazoline receptors.

But the 2-substituted quinoline fragment is present in the structure of the known antagonist of I_2-imidazoline receptors such as 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride (BU 224) [16]. It should be noted that N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) in terms of the theory of pharmacophore can be considered as a dimer BU 224, but it is not its dimer in terms of organic chemistry (Fig. 2).

According to the chemical structure, it can be assumed that N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) has the ability to interfere with the mechanisms of the carbohydrate metabolism regulation. There are data that this compound has antitumor properties *in vitro* due to enhanced apoptosis and activation of caspase-3 [9].

The aim of this work is to study the possible role of imidazoline receptors in the mechanism of the hypoglycemic action of N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide).

Materials and Methods

The study was conducted in white outbread mature male rats with the body mass of 0.20±0.02 kg. DM was modeled by subcutaneous injection of alloxan monohydrate solution (Sigma, USA) in a single dose of 150 mg/kg as 5% solution in acetate buffer, pH 4.5 [8]. Just this model is recommended as a basic one in pre-clinical studies of potential antidiabetic drugs [3]. The animals previously were fasted for 24 h, but had free access to water. In 10 days the rats with the basal glucose level higher than 11 mmol/l were selected [3, 4]. Glucose was determined in the blood samples taken from the vessels of tip of the tail by the glucose oxidase method using diagnostic kits ("Filicit", Ukraine).

The receptor mechanism of the hypoglycemic action was determined using efaroxan (Sigma, USA) as a blocker of I_1-imidazoline receptors in the dose of 5 mg/kg intraperitoneally [2], as well as 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride in the dose of 1.5 mg/kg intraperitoneally. The latter is known as Bu 224, which is I_2-imidazoline receptors selective blocker synthesized at the department of Organic Chemistry of the Kharkiv National University named after V.N. Karazin [6].

N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxami-

Table

The role of I_1- and I_2-imidazoline receptors in the mechanism of the hypoglycemic action of N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) administered intragastrically

Group of animals	n	Blood glucose, mmol/l		Decrease in the glucose level	
		basal level	in 90 min	absolute value	%
Intact control	12	3.50±0.21	3.37±0.26	0.13±0.37	+1.04±10.54
Diabetes (untreated)	6	20.32±1.92	21.47±2.22	1.15±0.79	+5.35±4.82
Diabetes + N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide), 11.64 mg/kg	9	25.41±2.53	7.39*±1.60	-18.03±1.98	-71.50**/*** ±4.82
Diabetes + (2-(4,5-dihydro-imidazol-2-yl)quinoline hydro chloride), 1.5 mg/kg + N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide), 11.64 mg/kg	10	26.25±2.28	12.02*±1.67	-14.23±1.97	-54.01**/***/**** ±5.62
Diabetes + efaroxan 5 mg/kg + N,N'-(ethane-1,2-dyyil) bis(quinoline-2-carboxamide), 11.64 mg/kg	9	27.30±3.23	14.18*±1.76	-13.12±2.88	-45.66**/***/**** ±8.15

Note: n – is the number of animals in the group; a statistically significant difference: * – with the basal value in the same group, $p<0.01$; ** – with the value of the intact control group, $p<0.01$; *** – with the untreated group value, $p<0.01$; **** – with the value of the group "diabetes + N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide), 11.64 mg/kg", $p<0.05$.

de) was administered as an aqueous suspension stabilized by polysorbate-80 in the dose of 11.64 mg/kg intragastrically being equal to ED_{50} calculated in the previous experiment [13], *per se*, or in 20 minutes after administration of imidazolin receptor blockers. The animals of the intact control group and the untreated diabetic group received an equivalent amount of the solvent (water for injections). The blood glucose level was determined before drug administration and in 90 min after that [3].

Statistical differences were analysed using Wilcoxon criterion Ť and Student t test [3].

Results and Discussion

N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) in the dose of 11.64 mg/kg intragastrically has an expressed hypoglycemic effect, significantly ($p<0.01$) reducing the level of blood glucose by 71.50% compared with the intact control group and the untreated group. On the background of (2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride – blocker of I_2-imidazoline receptors – the compound investigated decreases blood glucose levels by 54.01%, and it is significantly ($p<0.05$) less than the effect of the compound administered *per se*. Efaroxan that is I_1-imidazoline receptors blocker also prevented the hypoglycemic effect of N,N'-(ethane-1,2-dyyil)bis (quinoline-2-carboxamide), glycemia decrease was 45.66%, $p<0.05$ (Table).

The data obtained show the absence of significant differences between the degree of reduction in blood glucose on the background of both imidazoline receptors antagonists, indicating approximately equal participation of I_1- and I_2-imidazoline receptors in implementation of the hypoglycemic effect of N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide).

Thus, on the basis of pharmacological analysis it can be considered that the stimulation of both types of imidazoline receptors I_1 and I_2 is involved in the mechanism of the hypoglycemic action of N,N'-(ethane-1,2-dyyil)bis (quinoline-2-carboxamide) since specific blockers statistically significantly decrease its hypoglycemic effect.

CONCLUSIONS

1. It has been proven that N,N'-(ethane-1,2-dyyil) bis(quinoline-2-carboxamide) in the dose of 11.64 mg/kg has a significant hypoglycemic effect in alloxan-induced diabetic rats.

2. The hypoglycemic effect of N,N'-(ethane-1,2-dyyil) bis(quinoline-2-carboxamide) significantly decreases on the background of the specific antagonist of I_2-imidazoline receptors Bu 224 – 2-(4,5-dihydroimidazol-2-yl) quinoline hydrochloride – and on the background of I_1 receptor antagonist efaroxan. These data have proven the role of I_2- and I_1-imidazoline receptors stimulation in the mechanism of the hypoglycemic action of the compound investigated.

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ДОСЛІДЖЕННЯ РОЛІ ІМІДАЗОЛІНОВИХ РЕЦЕПТОРІВ I_1 ТА I_2 ТИПІВ У МЕХАНІЗМІ ГІПОГЛІКЕМІЧНОЇ ДІЇ N,N'-(ЕТАН-1,2-ДІЇЛ)БІС(ХІНОЛІН-2-КАРБОКСАМІДУ)

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Ключові слова: *N,N'-(етан-1,2-діїл)біс(хінолін-2-карбоксамід); гіпоглікемічна дія; імідазолінові рецептори; цукровий діабет; ефароксан; 2-(4,5-дигідроімідазол-2-іл)хінолін гідрохлорид (BU 224); алоксан*

Наведені результати дослідження механізму цукрознижувальної дії N,N'-(етан-1,2-діїл)біс (хінолін-2-карбоксаміду) при внутрішньошлунковому введенні у дозі 11,64 мг/кг на моделі алоксанового цукрового діабету у щурів. Досліджувана сполука з точки зору теорії фармакофорів може розглядатися як димер 2-(4,5-дигідроімідазол-2-іл)хіноліну гідрохлориду, блокатора імідазолінових рецепторів типу I_2, відомого під шифром BU 224, але не є його димером з точки зору органічної хімії. Є дані, що ця сполука має протипухлинні властивості in vitro за рахунок посиленого апоптозу та активації каспази-3. Відомості щодо впливу досліджуваної сполуки на вуглеводний обмін обмежені. Виходячи з хімічної будови, а саме наявності 2-заміщеного хінолінового фрагменту, характерного для 2-(4,5-дигідроімідазол-2-іл)хінолін гідрохлориду, висунуто припущення, що N,N'-(етан-1,2-діїл)біс(хінолін-2-карбоксамід) має здатність втручатися в механізми регуляції вуглеводного обміну. Отримані результати свідчать,що при алоксановому діабеті N,N'-(етан-1,2-діїл)біс(хінолін-2-карбоксамід) чинить виразну цукрознижувальну дію, знижуючи рівень глікемії на 71,50%, та є агоністом імідазолінових рецепторів обох типів, що було доведено шляхом їх блокади селективними антагоністами – ефароксаном та 2-(4,5-дигідроімідазол-2-іл)хіноліну гідрохлоридом. На фоні блокатора імідазолінових рецепторів типу I_1 ефароксану зниження глікемії становило 45,66% ($p<0,05$), а на тлі блокатора імідазолінових рецепторів типу I_2 2-(4,5-дигідроімідазол-2-іл)хінолін гідрохлориду – 54,01% ($p<0,05$).

ИССЛЕДОВАНИЕ РОЛИ ИМИДАЗОЛИНОВЫХ РЕЦЕПТОРОВ I_1 И I_2 ТИПОВ В МЕХАНИЗМЕ ГИПОГЛИКЕМИЧЕСКОГО ДЕЙСТВИЯ N,N'-(ЭТАН-1,2-ДИИЛ)БИС(ХИНОЛИН-2-КАРБОКСАМИДА)

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Ключевые слова: *N,N'-(этан-1,2-диил)бис(хинолин-2-карбоксамид); гипогликемическое действие; имидазолиновые рецепторы; сахарный диабет; эфароксан; 2-(4,5-дигидроимидазол-2-ил)хинолин гидрохлорид (BU 224); аллоксан*

Приведены результаты исследования механизма сахароснижающего действия N,N'-(этан-1,2-диил)бис(хинолин-2-карбоксамида) при внутрижелудочном введении в дозе 11,64 мг/кг на модели аллоксанового сахарного диабета у крыс. Исследуемое соединение с точки зрения теории фармакофоров может рассматриваться как димер 2-(4,5-дигидроимидазол-2-ил)хинолин гидрохлорида, блокатора имидазолиновых рецепторов типа I_2, известного под шифром BU 224, но не является его димером с точки зрения органической химии. Есть данные, что это соединение имеет противоопухолевые свойства in vitro за счет усиленного апоптоза и активации каспазы-3. Сведения о влиянии исследуемого соединения на углеводный обмен ограничены. Исходя из химического строения, а именно наличия 2-замещенного хинолинового фрагмента, характерного для 2-(4,5-дигидроимидазол-2-ил)хинолин гидрохлорида, выдвинуто предположение, что N,N'-(этан-1,2-диил)бис(хинолин-2-карбоксамид) обладает способностью вмешиваться в механизмы регуляции углеводного обмена. Полученные результаты свидетельствуют, что при аллоксановом диабете N,N'-(этан-1,2-диил)бис(хинолин-2-карбоксамид) оказывает выраженное сахароснижающее действие, снижая уровень гликемии на 71,50%, и является агонистом имидазолиновых рецепторов обоих типов, что было доказано путем их блокады селективными антагонистами – эфароксаном и 2-(4,5-дигидроимидазол-2-ил)хинолин гидрохлоридом. На фоне блокатора имидазолиновых рецепторов типа I_1 эфароксана снижение гликемии составило 45,66% ($p<0,05$), а на фоне блокатора имидазолиновых рецепторов типа I_2 2-(4,5-дигидроимидазол-2-ил)хинолин гидрохлорида – 54,01% ($p<0,05$).