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

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: $I_1$ and $I_2$. 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.
But the 2-substituted quinoline fragment is present in the structure of the known antagonist of I₂-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 outbred 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 I₁-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 I₁-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-carboxamide)

| Group of animals | n | Blood glucose, mmol/l | Decrease in the glucose level |
|------------------|---|-----------------------|-------------------------------|
|                  |   | 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-dihydroimidazol-2-yl)quinoline hydrochloride), 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.
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$_1$-imidazoline receptors Bu 224 – 2-(4,5-dihydroimidazol-2-yl) quinoline hydrochloride – and on the background of I$_2$ receptor antagonist efaroxan. These data have proven the role of I$_1$- and I$_2$-imidazoline receptors stimulation in the mechanism of the hypoglycemic action of the compound investigated.

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ДОСЛІДЖЕННЯ РОЛІ ІМІДАЗОЛІНОВИХ РЕЦЕПТОРІВ I, TA І2 ТИПІВ У МЕХАНІЗМІ ГІПОГЛІКЕМІЧНОЇ ДІЇ N,N’-(ЕТАН-1,2-ДІІЛ)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-карбоксамід) 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, 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 углеводний обмін обмежені. Виходячи з хімічної будови, а саме наявності 2-заміщеного хінолінового фрагменту, характерного для 2-(4,5-дигідроімідазол-2-іл)хінолінів, висунуто припущення, що N,N’-(етан-1,2-діїл)біс(хінолін-2-карбоксамід) має здатність втручатися в механізмі регуляції углеводного обміну. Отримані результати свідчать, що при алоксановому діабеті N,N’-(етан-1,2-діїл)біс(хінолін-2-карбоксамід) чинить вплив на гіпоглікемічну дію, знижуючи рівень глікемії на 71,50%, та є агоністом імідазолінових рецепторів обох типів, що було доказано їх блокадою селективними антагоністами – ефароксаном та 2-(4,5-дигідроімідазол-2-іл)хіноліну гідрохлориду. На фоні блокатора імідазолінових рецепторів типу I, ефароксану, зниження глікемії становило 45,66% (p<0,05), а на тлі блокатора імідазолінових рецепторів типу I2, вуглеводний обмін, вони діють на рівні 54,01% (p<0,05).