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ION CHANNEL TRPA₁ IS A PROMISING THERAPEUTIC TARGET FOR TREATMENT OF PAIN

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Abstract. TRPA₁ is an ankyrin receptor of TRP family. It is a non-selective, calcium-permeable cation channel that is highly expressed by a subset of small diameter sensory neurons with cell bodies in the dorsal root, trigeminal, nodose and jugular ganglia. In the last time information about the role of TRPA₁ in pain and cold sensitivity, as well as in the formation and maintenance of inflammation is increasing in scientific literature. Given this information, the interest for search and study of pharmacological agents, which selectively blocked of TRPA₁ and reduced the severity of pain and inflammation is increasing.

Keywords: ion channel TRP; TRPA₁; pain; ketorolac, nociception; analgesia; neuropathy.

Pain is a complaint for appeal in the primary healthcare departments, which mainly the load of the treatment of patients with different types of pain syndromes. Besides, costs of treating chronic pain and acute pain relief steadily increase. It is known that about 90% of all diseases are associated with pain [7].

International epidemiological study showed that 49.2% patients of primary healthcare departments had complaint of pain. In according to European epidemiological study 19% of adult Europeans suffer from pain of moderate to severe intensity, which reduces the quality of life, breaking the everyday routine and work. About half of such patients have not received appropriate treatment [4].

According to the Russian Association for the study of pain the prevalence of chronic pain in Russia is average 34.3 cases per 100 persons. More than 40% patients with chronic pain say that pain reduces significantly the quality of their life [1, 2].

Among the approaches used to adjust the use of molecular methods for screening, at the cellular, organ, and organism levels integral.

Today narcotic analgesics and non-steroid anti-inflammatory drugs use for treatment of pain more often. However, both of it have many side effects that limit its widespread and long term use in clinical practice.

Thus, today pharmaceutical market doesn't have analgesic drugs that completely satisfy the requirements of efficiency and safety. That's why the most promising solution is to provide new analgesic drug based on selective inhibition of TRPA₁.

TRPA₁ is highly expressed by a subset of small diameter sensory neurons with cell bodies in the dorsal root, trigeminal, nodose and jugular ganglia and immediately perceives painful stimuli and inflammatory mediators [6, 8]. The results of their research some authors have reported an increase in expression of TRPA₁ in pathological conditions, for example, inflammation, neuropathy [3, 5]. Today data is increasingly appearing in the literature on the

possible use of TRPA₁ selective inhibitors for treatment of pain syndrome.

The goal of research is study of the analgesic activity of a selective inhibitor of TRPA1 receptors compared with the reference drug ketorolac.

Materials and methods. Experiments were performed on male white laboratory mice weighing 20-22 g. Study of the analgesic activity of TRPA₁ selective inhibitor was performed in the test «hot plate» in intragastric and intramuscular routes of administration. Ketorolac was used as a comparison drug. Intact animals were placed on a heated to 55 °C plate and fixed the time in seconds before the first lick their paws.

All the animals were divided into 3 groups of each 2 series:

Group I (control): series 1 – intragastric administration of placebo (normal saline) in a volume of 0.1 ml / 10 g body weight (n = 20).

Series 2 – intramuscular injection of placebo (normal saline) in a volume of 0.1 ml / 10 g body weight (n = 20).

Group II: series 1 – intragastric administration of ketorolac in a dose of 3.48 mg / kg (n = 20).

Series 2 – intramuscular injection of ketorolac in a dose of 3.48 mg / kg (n = 20).

Group II: series 1 – intragastric administration of TRPA₁ selective inhibitor (ZB-010621 substance) in a dose of 3 mg / kg (n = 20).

Series 2 – intramuscular injection of TRPA₁ selective inhibitor (ZB-010621 substance) in a dose of 3 mg / kg (n = 20).

Then, at 30, 60, 90 and 120 minutes after the administration of substances the animals were placed again at the hot plate and the time in seconds was fixed before the start of the animal licking paws.

Data adequacy of the study were determined by methods of descriptive and analytical statistics with the definition of arithmetical mean (M), their standard mean square error ($\pm m$) and P-value (p), calculated using the T-test for groups with different dispersion. Differences were estimated as significant at $p < 0.05$. Statistical calculations were performed using SPSS 21 software.

Results. In intragastric administration of the test substances has been found that a dose of ketorolac 3.48 mg/kg, and TRPA₁ selective inhibitor (ZB-010621 substance) in a dose of 3 mg/kg exhibit its greatest analgesic activity after 60 minutes from the time of administration (Table 1).

Table 1

The results of «hot plate» test on male white laboratory mice (M $\pm$ m)

Time	Control group		Ketorolac group		ZB-010621 substance group	
	intragastric	intramuscular	intragastric	intramuscular	intragastric	intramuscular
Depature	7.93 $\pm$ 0.23	7.7 $\pm$ 0.67	7.42 $\pm$ 0.49	6.10 $\pm$ 0.81	7.60 $\pm$ 0.52	8.21 $\pm$ 0.81
30 min	8.54 $\pm$ 0.72	9.25 $\pm$ 0.92	13.57$\pm$0.59*	16.41$\pm$1.01*	18.81$\pm$0.76**	22.19$\pm$1.12**
60 min	9.5 $\pm$ 0.88	9.73 $\pm$ 0.9	15.91$\pm$0.61*	13.74$\pm$1.16*	21.7$\pm$0.82**	19.57$\pm$1.43**
90 min	9.75 $\pm$ 0.90	8.4 $\pm$ 0.71	12.56 $\pm$ 0.55	11.82 $\pm$ 1.37	17.63$\pm$0.79**	17.44$\pm$1.79**
120 min	9.39 $\pm$ 0.90	8.2 $\pm$ 0.70	8.34 $\pm$ 0.54	9.21 $\pm$ 0.92	13.75$\pm$0.71**	14.78$\pm$0.92**

Note: * - significant difference from control group ($p < 0.05$), ** - significant difference from Ketorolac group ($p < 0.05$).

However, TRPA₁ selective inhibitor (ZB-010621 substance) showed a pronounced analgesic action compared to ketorolac (21.7 $\pm$ 0.82 sec and 15.91 $\pm$ 0.61 sec, respectively). Moreover, in the group of ketorolac at 90 minutes from the introduction the arithmetical mean of time from the animals were placed on a heated to 55 °C plate till the moment licking paws were not significantly different from the control group (12.56 $\pm$ 0.55 sec and 9.75 $\pm$ 0.90, respectively), whereas the analgesic effect of a TRPA₁ selective inhibitor (ZB-010621 substance) lasted for 90 minutes, and 120 minutes following administration of substance (17.63 $\pm$ 0.79 seconds and 13.75 $\pm$ 0.71 sec respectively, $p < 0.05$ compared with ketorolac).

In intramuscular administration of the test substances ketorolac and TRPA₁ selective inhibitor

(ZB-010621 substance) exhibit analgesic activity maximum at 30 minutes after administration (16.41 $\pm$ 1.01 sec and 22.19 $\pm$ 1.12 respectively). However, TRPA₁ selective inhibitor (ZB-010621 substance) exceeds ketorolac not only in the expression of analgesic activity, but also its duration, as evidenced by the longer time from the moment from the animals were placed on a heated to 55 °C plate till the moment licking paws in the group of TRPA₁ selective inhibitor (ZB-010621 substance) compared with the control group after 60, 90 and 120 min, while the action of ketorolac by the ends 90 minutes (Table 1).

Conclusions. Thus, TRPA₁ selective inhibitor (ZB-010621 substance) in the «hot plate» test exceeds ketorolac not only in the expression of analgesic activity, but also the duration of its as in

intragastric and intramuscular administration of test substances.

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