Analgesic Activity of Diterpene Alkaloids from *Aconitum Baikalensis*


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We compared analgesic activities of individual alkaloids extracted from Baikal aconite (*Aconitum baikalensis*): napelline, hypaconitine, songorine, mesaconitine, 12-epinapelline N-oxide. The detected analgesic activity was comparable to that of sodium metamizole. The mechanisms of analgesia were different in diterpene alkaloids of different structure. The antinociceptive effect of atisine alkaloids (12-epinapelline N-oxide, songorine) was naloxone-dependent and realized via opioid receptor modulation.

Key Words: *Aconitum baikalensis*; diterpene alkaloids; analgesic activity

About 24% of all research carried out all over the world is aimed at the search for new substances with analgesic activity [1]. Great interest to the problem is explained by the fact that 90% diseases are associated with pain and about 20% of the population of the Earth suffer from chronic pain. More than 30 million people daily use an analgesic all over the world, but this “treatment” is one of the most typical causes of untoward reactions to drug therapy.

Alkaloids, regulating many vital processes in the organism, are prospective sources of new drugs [2]. Our studies detected various physiological activities in diterpene alkaloids extracted from *Aconitum baikalensis*: stress-protective [8], antimetastatic [7], regenerative [12,14], antidepressant [11], anti-inflammatory [3,5,6], antihypoxic, antipyretic [4].

We studied the analgesic activities of diterpene alkaloids individually extracted from the Baikal aconite.

MATERIALS AND METHODS

The study was carried out on outbred adult CD1 and CBA mice (20-30 g) and outbred rats (200-300 g).

First-category animals from Department of Experimental Biological Models of E. D. Goldberg Research Institute of Pharmacology were kept under standard vivarium conditions with 12:12 h day:night regimen on balanced diet (GOST R 50258-92) with free access to water and food. Experiments were carried out with due consideration for the International Recommendations of the European Convention for Protection of Vertebrates Used in Experimental Studies (1998) and Laboratory Practice Regulations for Preclinical Studies in the Russian Federation (GOST R 51000.3-96 and GOST R 51000.4-2008). The animals were sacrificed in accordance with the Regulations of Studies with the Use of Experimental Animals, approved by the Ministry of Health of Russia. The objects of the study were individual diterpene alkaloids (mesaconitine, hypaconitine, napelline, songorine, 12-epinapelline N-oxide) extracted from raw *Aconitum baikalensis* terrestrial part (grass) at laboratory of Irkutsk Institute of Organic Chemistry. The alkaloids were extracted from raw material (grass) by chloroform extraction in the form of free bases, separated, and identified by the standard methods. Analgin (sodium metamizole), a well-known analgesic, served as the reference drug (250 mg/kg). *Aconitum baikalensis* alkaloids (0.0063, 0.0125, 0.025, and 0.05 mg/kg) and the reference drug...
were injected during 5 days, the last dose was injected 1 h before exposure. Controls received the solvent in an equivalent volume.

Studies of the pharmacological activities of bioactive compounds and the choice of the reference drug were carried out in accordance with the recommendations on preclinical studies of new anti-inflammatory drugs [9].

The specific painful reaction (cramp) was induced in mice by intraperitoneal injection of 0.75% acetic acid (GOST 61-75; 0.1 ml/10 g) 1 h after the last injection of the studied substances. The cramps (convulsions) were counted over 15 min after injection in each animal and the latent time before their manifestation was recorded. Another chemical algogenic substance used in the study was acetylcholine hydrochloride, which was also injected intraperitoneally (0.1 ml, 0.3 mg/10 g). The painless reaction (cramps) were recorded directly after its injection during 5 min. Drug efficiency in both experiments was evaluated by the difference in the mean number of cramps in the control and experimental groups.

The analgesic activity under conditions of mechanical painful irritation was evaluated on the immune inflammation model by the number of vocalization episodes during passive bending (5 times) of the arthritic talocrural joint in control and experimental mice. Arthritis was simulated by injection of Freund’s complete adjuvant (BCG suspension 2.5 mg/ml in mineral oil). The phlogogen was injected to rats under the hind right paw pad in a single dose of 0.1 ml. The severity of the painful reaction was evaluated by the vocalization index, calculated by the formula:

\[ V = \frac{N}{5} \times 100\% \]

where N was the number of vocalization episodes.

In order to evaluate the involvement of the opioid system in the mechanisms of analgesic activities of the diterpene alkaloids, the analgesic activity of these compounds under conditions of chemical painful stimulation (acetylcholine cramps) was evaluated after injection of naloxone (opioid receptor antagonist) [1]. Naloxone (Sigma) was injected subcutaneously (1 mg/kg) 50 min after all the studied agents and 10 min before reproduction of the acetylcholine cramp model. It was assumed that if naloxone injection arrested analgesia induced by the preinjected test substance – this substance was tropic to opioid receptors and its effect was naloxone-dependent. If naloxone failed to induce this effect – the agent was not naloxone-dependent and not an opioid receptor system ligand.

The results were processed by variational statistical methods with Student’s t test and Mann–Whitney nonparametric U test using Statistica 6.0 software. The differences were considered significant at \( p \leq 0.05 \).

RESULTS

The latency of painful reaction in the acetic cramps model increased by 2.8-3.3 times under the effect of all the studied alkaloids (Fig. 1, b), while the number of cramps was by 52-65% below the control (Fig. 1, a). Hypaconitine exhibited the highest activity in this experiment.

The period before the onset of convulsions in mice injected with the alkaloids in doses of 0.025, 0.0125, and 0.0063 mg/kg was longer by 3.4-5.3 times in response to napelline, by 1.7-3.4 times longer after songorine, by 2.0-3.2 times after hapaconitine, and by 2.2-2.3 times after 12-epinapelline N-oxide, while the number of convulsions decreased by 65-89% after napelline, by 69-79% after hapaconitine, and by 47-57% after 12-epinapelline N-oxide. Sodium met-

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**Fig. 1.** Analgesic effects of alkaloids individually extracted from *Aconitum baikalensis* under conditions of acetic cramps model in mice. a) Number of convulsions; b) latent period of painful reaction. C) Control; 1) songorine; 2) napelline; 3) mesaconitine; 4) hypaconitine; 5) 12-epinapelline N-oxide. *p<0.05 in comparison with the control.