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NEURO- AND PSYCHOPHARMACOLOGICAL INVESTIGATION OF THE ALKALOIDS CONVOLVINE AND ATROPINE

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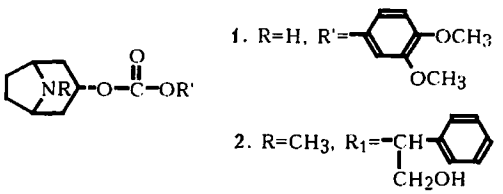
It has been established that convolvine blocks the M-receptors of the heart and intestine but raises the sensitivity of the M-receptors of the salivary gland and of the CNS, while atropine blocks all the above-mentioned M-receptors. Convolvine has revealed characteristics of a sedative and nootropic agent. Atropine, however, which is known as a psychomotor stimulator, prevents the realization of a conditioned gastromotor reflex. An analysis has been made of the relationship between features of the pharmacological activities and chemical structures of convolvine and atropine.

Plants of the genus *Convolvulus* (bindweed) were first studied by A. P. Orekhov, R. A. Konovalova [1], and S. Yu. Yunusov. From *C. pseudocantabrica* and *C. subhirsutus* they isolated the two alkaloids convolvine and convolamine and established their structures. They were the first to show the presence of tropane alkaloids in plants of the Convolvulaceae family. S. Yu. Yunusov and coworkers determined the maximum amounts of the total alkaloids in the epigeal parts of the plants — 2.08% — and in the roots — 4.1% [2].

The main alkaloid of both species is convolvine, making up about 1% of the air-dry weight of the raw material. Convolvine (**1**) is an ester of the amino alcohol nortropine and 3,4-dimethoxybenzoic (veratric) acid and belongs to the class of tropane alkaloids, like atropine (**2**), which is an ester of the amino alcohol tropine and *dl*-tropic acid. Atropine is one of the standard M-cholinoblockers. With the aim of evaluating the neuro- and psychotropic activities of convolvine and also to elucidate the relationship between structural features and biological activity we have made a comparative study of convolvine and atropine in those directions where the pharmacological properties of the latter are generally known.



In control experiments on anesthetized rats, the M-cholinostimulator carbachol in a dose of 0.02 mg/kg i/v lowered the frequency of cardiac contractions from 384 ± 24 to 68 ± 17 beats per minute and also caused the secretion of 0.86 ± 0.18 g/kg of saliva. In small doses, atropine prevented the above-mentioned effects of carbachol, its ED_{50} values being 0.011 and 0.008 mg/kg i/v, respectively. Convolvine decreased the degree of expression of bradycardia with an ED_{50} of 1.8 mg/kg i/v, while salivation under the action of convolvine, administered in doses reaching the sublethal level, increased it by up to 76%. Both atropine and convolvine decreased intestinal spasm caused by carbachol, their EC_{50} values being $1.4 \cdot 10^{-9}$ and $4.3 \cdot 10^{-7}$ g/ml, respectively. The antagonism of convolvine to barium chloride spasm amounted to $2 \cdot 10^{-6}$ g/ml, i.e., it was approximately 5 times less pronounced than in relation to carbachol spasm.

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The influence of these substances on the M-cholinoreceptors of the CNS was investigated in a test of their action on arecoline tremor. The experiment showed that in a dose of 5 mg/kg i/p convolvine shortened the latent period of the setting in of this effect from 141 ± 16 to 106 ± 12 sec, i.e., by 25%. The duration of the tremor increased from 605 ± 125 to 986 ± 148 sec, or by 63%. In a dose of 5 mg/kg i/p, the classical M-cholinoblocker atropine eliminated arecoline tremor completely.

In experiments on rats, convolvine in doses of 3.0 and 10 mg/kg s/c prolonged the hypnotic effect of ethaminal sodium from 58 ± 16 to 95 ± 19 and 107 ± 22 min (by 64 and 84%), respectively.

In a test on the aggressiveness of white mice caused by mechanical restraint, convolvine in doses of 1.0 and 5 mg/kg i/p lowered the number of bites by 24 and 49%, respectively. In the same doses, atropine had no influence on the feeling of aggressiveness in mice.

The effects of convolvine and atropine on the rate of realization of the induced gastromotor reflex of rats were not the same. In a dose of 1.0 mg/kg convolvine exhibited a tendency to shorten the time of realization of the reflex from 8.2 ± 2.4 to 6.5 ± 1.8 sec (21%), and in a dose of 5.0 mg/kg i/p from 9.4 ± 3.1 to 6.3 ± 1.5 sec (33%). At the same doses, atropine lenthened the time of realization of the reflexes from 8.8 ± 1.8 to 18.9 ± 4.7 and 33.5 sec, or 2.1- and 3.8-fold.

A comparative neuro- and psychopharmacological investigation revealed a moderate M-cholinoblocking activity of convolvine on the heart and intestine, amounting to 0.6 and 0.3%, respectively, of the activity of atropine. In addition to this, a substantial rise in the sensitivity of the M-receptors of the salivary glands and of the CNS to the action of carbachol and arecoline was observed. Atropine, however, behaved as a highly active M-cholinoblocker in all the experiments. The lengthening of hypnotic effect of ethaminal sodium by about 84% and the decrease in the aggressive action in mice by about 49% caused by convolvine can be interpreted as a sedative property of convolvine, while a shortening by 33% of the time for rats to run through a maze can be interpreted as a nootropic effect. However, atropine, which is known as a psychomotor stimulator, did not affect aggressiveness in mice and prolonged the time of maze-running by a factor of about 4.

On the basis of modern results on the classification of muscarinic receptors [3], convolvine may be taken as a mixed $M_{2,4}$-cholinoblocker free from M_3-blocking properties.

In spite of the fact that convolvine and atropine belong to the same class of tropane alkaloids, the results of the investigation witness that these substances cannot be assigned unconditionally to the same pharmacological class of biologically active compounds.

In a comparison of the chemical structures of atropine and convolvine it may be noted that the compounds have the same alcoholic moiety, while there are substantial differences in the acid components. In atropine the acid residue includes a primary alcoholic hydroxy linked to a methine group, which is important for the manifestation of its specific activity. In the first place, this grouping lengthens the binding element between the alcoholic and acidic moieties and makes it correspond in an optimum degree to an M-cholinoreceptor, which is confirmed by the extremely high activity of atropine as an M-cholinoblocker, and, in the second place, the presence of this grouping makes the link between the alcoholic and acidic parts more mobile, thereby ensuring a greater adaptability of atropine to the M-receptors of various organs belonging to different subtypes (M_1-M_4) [3]. In convolvine, however, in view of the absence of this element, the distance between the alcoholic and acidic moieties of the molecule is shortened; furthermore another important element — the alcoholic hydroxyl, which takes part in binding with a muscarinic receptor [4] — has disappeared. These differences are responsible for the low muscarinoblocking activity of convolvine and its lower adaptability to all subtypes of M-receptors.

The two methoxy groups in the acid part of the molecule increase its weight, give it greater inertia, and, possibly, weaken its relative selectivity for M_{2-4}-receptors. Even in sublethal doses, convolvine exhibits no muscarinolytic action; however, it raises the sensitivity of several subtypes of M-receptors to the action of M-stimulators, as has appeared in the salivary glands, where the M_3-subtype predominates and an enhancement of carbachol salivation has been observed.

A matter of definite interest is the potentiation by convolvine of the effects of the central M-cholinostimulator arecoline, which, according to recent results, can be used to improve the mental capacities of elderly people suffering from Alzheimer's disease [5]. Central selective M_2-cholinoblockers, to which convolvine may also be assigned, are likewise included among the promising drugs for the treatment of this disease [6]. On the basis of the facts given above and bearing in mind the element of nootropic action of convolvine that we have established previously, it may be concluded that further investigations of the influence of this alkaloid on cognitive properties are promising within the framework of the search for new psychotropic agents improving mental activity.

EXPERIMENTAL

The M-receptors of the CNS in white mice were stimulated by the injection of arecoline in a dose of 10 mg/kg i/p, which caused tremor in 100% of the animals. The latent period of the beginning of the tremor and its duration in seconds were recorded. Stimulation of the M-receptors of the heart and the salivary glands was carried out in experiments on narcotized rats (ethaminal sodium, 40 mg/kg i/p) with the aid of an injection of carbachol in a dose of 0.02 mg/kg i/v, which is the maximum tolerated by narcotized rats. The influence of the substances on the M-receptors of the intestine was studied on isolated rat ileum using Tyrode's solution at 37°C. Spasm of the organ was evoked by carbachol in concentrations of $5 \cdot 10^{-8}$, 10^{-7}, and $2 \cdot 10^{-7}$ g/ml or barium chloride at 10^{-4}, $2 \cdot 10^{-4}$, and $4 \cdot 10^{-4}$ g/ml, causing a spasm reaching a degree of expression 80-90% of the possible.

The aggressiveness of white mice was evoked by the method of G. C. Wagner [7] with the aid of mechanical restraint in a narrow glass cylinder at the end of which there was a target consisting of two plate-type electrodes the biting of which closed an electric circuit with a recorder. An increase in the number of bites showed an increase in aggressiveness and conversely. The influence of the substances on the rate of realization of the gastromotor conditioned reflex was investigated in experiments on rats deprived of food for 24 h. In preliminary experiments the rats were trained to find food in a maze, and the influence of the substances on the speed of running the maze was evaluated.

The isolation of convolvine from *C. subhirsutus* was achieved as described previously [8].

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