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STRUCTURE AND M-CHOLINOBLOCKING ACTIVITY OF CHLORINE DERIVATIVES OF IMPERIALINE

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Characteristics of the M-cholinoblocking activities of the alkaloid imperialine, three of its chlorine derivatives, and atropine have been shown. α-Chloroimperialine is a highly active central mixed $M_{2,4}$-cholinoblocker, while its β-isomer is predominantly a peripheral mixed $M_{2,4}$-cholinoblocker; a mixture of the α- and β-isomers is a central selective, and imperialine a peripheral selective, M_2-cholinoblocker.

Among the neuroreceptors of the organism, the muscarinic receptors are the most widely distributed and take part in the regulation of the activity of all the vitally important organs [1]. It was previously considered that the muscarinergic system was a unity. Of the group of muscarinotropic agents, the M-cholinoblocker atropine and substances similar to it are used in cardiology, gastroenterology, ophthalmology, anesthesiology, etc. In view of the fact that these substances block the M-receptors of various organs simultaneously, their therapeutic use is accompanied by many side-effects, and the search for selective M-cholinotropic agents is an urgent task in pharmacology.

In recent years proofs have appeared of the existence of four subtypes of M-cholinoreceptors [2]. The process of differentiating M-receptors is obviously still incomplete. The clinical use of selective M-cholinotropic substances is in its initial stage. It is known that the M_1-cholinoblocker pirenzepine or gastrozepin is being used successfully for the treatment of stomach and duodenal ulcers [3]. The possibility has been suggested of using selective M-cholinotropic compounds in the treatment of Alzheimer's disease [4].

It has been established previously that imperialine and a number of its esters possess a selective M_2-cholinoblocking property [5-8]. In this paper we give the results of an investigation of features of the cholinoblocking activity of chlorine derivatives of imperialine: α-chloroimperialine, β-chloroimperialine, and a mixture of the α- and β-isomers (α,β-chloroimperialine).

We isolated imperialine (**1**) from the bulbs and epigeal parts of a plant of the *Petilium* genus [9], and from it obtained a mixture of α- and β-chloroimperialines, the subsequent separation of which led to α-chloroimperialine (**2**) and β-chloroimperialine (**3**). The structures of (**2**) and (**3**) were established on the basis of spectral characteristics [8]. We carried out a comparative investigation of the chlorine derivatives with atropine and imperialine. We investigated the antagonism of the compounds to the action on the heart of the negative chronotropic action of carbachol administered in the maximum tolerated dose, 0.02 mg/kg i/v, to narcotized rats. In these experiments we evaluated the M_2-cholinoblocking activities of the compounds. The antagonism of the compounds to the secretory action of carbachol was taken as M_3-cholinoblocking activity; antagonism to the spasmogenic action of carbachol on isolated ileum as M_4-cholinoblocking activity; and antagonism of the alkaloids to the tremor action of arecoline as the central M-blocking activity.

We calculated the doses of the substances lowering the effects of carbachol and arecoline by 50%, i.e., ED_{50} and EC_{50}. The activity indices were expressed in micromoles — μmole/kg and μmole/liter —, and also in percentages of the activity of the standard M-cholinoblocker atropine sulfate. As the results of the investigation (Table 1) show, α-chloroimperialine proved to be the most active, and its β-isomer the least active. The individual α- and β-chloroimperialines lost their selectivity for the M-cholinoreceptors of the heart and intestine, relating to the M_2- and M^4-subtypes, respectively, since the difference in their activities was less than one order of magnitude. At the same time, the mixture of α- and β-isomers retained its selectivity between the above-mentioned subtypes of M-cholinoreceptors. It follows from this that the mixture of isomers, as also the alka-

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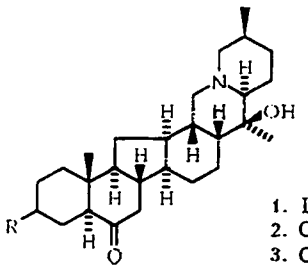
TABLE 1. Indices of the Peripheral and Central M-Cholinoblocking Activities of Atropine and of Imperialine and Its Chloro- Derivatives on Organs

Substance	ED_{50} and EC_{50} values of the M-cholinoblocking activities on the organs				
	heart, μmole/kg (M_2)	intestine, μmole/liter (M_4)	salivary glands (M_3)	ratio between M_2, M_4, and M_3	M-receptors of the CNS, μmole/kg
1. Atropine	0.031±0.012 (100%)	0.0028±0.0014 (100%)	0.016±0.007 (100%)	1:1:1	6.7±2.2 (100%)
2. Imperialine	0.124±0.062 (25%)	0.52±0.24 (0.54%)	26.2±12.4 (0.057%)	438:25:1	32.7±7.1 (20.5%)
3. α,β-Chloroimperialine	0.012±0.005 (258%)	0.028±0.012 (10%)	1.45±0.62 (1.1%)	258:10:1	0.57±0.22 (527%)
4. α-Chloroimperialine	0.008±0.004 (388%)	0.002±0.001 (127%)	0.16±0.07 (10%)	39:13:1	0.18±0.08 (1675%)
5. β-Chloroimperialine	0.026±0.009 (119%)	0.013±0.006 (22%)	0.62±0.24 (2.6%)	46:8:1	7.4±2.9 (91%)

loid imperialine, can be called a selective M_2-cholinoblocker, while in the individual form the isomers are mixed $M_{2,4}$-cholinoblockers. In evaluating the (nonspecific) central M-cholinoblocking activities of the compounds under discussion it must be borne in mind that they all, to a greater or smaller degree, penetrate through the blood-brain barrier, while α-chloroimperialine, a central M-cholinolytic, is 17 times superior to atropine in antitremor activity and is followed by the mixture of α- and β-isomers (5.3 times); the β-isomer proved to be less active (roughly equal to atropine), and the least active was the alkaloid imperialine (20% of the activity of atropine).

On an attentive consideration of features of the M-cholinoblocking properties of the chlorine derivatives it can be noted that with respect to M_2-blocking activity on the heart (see Table 1) α,β-chloroimperialine occupies an intermediate position between the individual α- and β-isomers. So far as concerns M-blocking activity on the intestine (M_4) and on the salivary gland (M_3), here the mixture of isomers is considerably less active than the individual isomers. The impression is created that the component parts of the mixture exhibit a definite antagonism to one another in their blocking action on the above two types of M-receptors. Without entering into a discussion on the subject of possible mechanisms for the phenomena mentioned, we must come to the conclusion, important from the practical point of view, that the mixture of isomers combines in itself the selectivity for the M_2-subtype of receptors characteristic of the known M_2-blocker imperialine, high activity, and good penetrability through the blood-brain barrier (5 times more actively than atropine). These properties permit the conclusion that the mixture of isomers may be used a tool for the selective blockade of the central M_2-cholinoreceptors, and the α- isomer as a central $M_{2,4}$-cholinoblocker; provisionally, the β-isomer may be taken as a peripheral $M_{2,4}$-cholinoblocker.



1. Imperialine R=β OH
2. Chloroimperialine R=α Cl
3. Chloroimperialine R =β Cl

EXPERIMENTAL

The isolation of a mixture of α- and β-chloroimperialines and the separation of compounds (2) and (3) have been described previously [8].

Stimulation of the M-cholinoreceptors of the heart and salivary glands was achieved by the i/v injection of carbachol in a dose of 0.02 mg/kg into narcotized rats (pentobarbital sodium, 40 mg/kg, i/p). This dose of carbachol was the maximum tolerated by the narcotized rats. In the experiments we recorded the influence of the substances on the bradycardia caused by the maximum tolerated dose of carbachol. In control experiments the action of the given dose of carbachol lowered the frequency of cardiac contractions from 382 ± 18 to 74 ± 12 beats per minute; i.e., the level of bradycardia was 308 beats/-

min. The doses of the substances decreasing the level of bradycardia by 50% were calculated by a graphical method. In the same experiments we calculated the ED_{50} values of the substances decreasing salivation by 50%. The amount of saliva was measured on a torsion balance. It was collected for 3 min after the administration of carbachol with the aid of a piece of hygroscopic bandage weighing 80-100 mg placed in the oral cavity. The influence of the substances on the M_4-receptors was tested in experiment on rat ileum, isolated by the Magnus method, using Tyrode solution at 37°C with aeration. The M-receptors of the ileum were stimulated with the aid of carbachol in concentrations of $5 \cdot 10^{-8}$, 10^{-7}, and $2 \cdot 10^{-7}$ g/ml, giving spasms with a magnitude of 80-90% of the maximum possible. The M-receptors of the CNS were stimulated by means of arecoline administered in a dose of 10 mg/kg i/p, giving tremor in 100% of the animals.

The statistical treatment was performed by the Factorp-Pedersen method at $P = 0.05$.

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