DEPENDENCE OF THE M-CHOLINOMIMETIC PROPERTIES OF ARECOLINE ANALOGS ON THE STRUCTURAL PECULIARITIES OF THEIR MOLECULES

N. I. Kudryashova and N. A. Losev

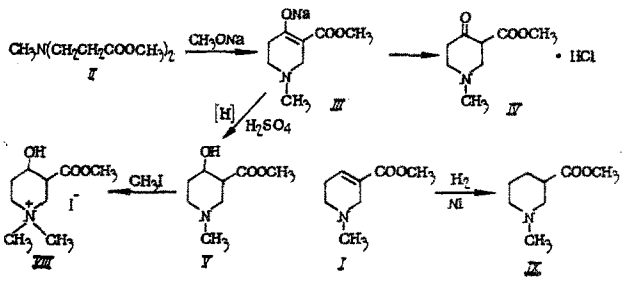
UDC 615.217.32:577.2

Arecoline (I) produces a pronounced M-cholinomimetic effect, and, as a tertiary lipid-soluble amine, it penetrates well through the blood-brain barrier, exerting a stimulating influence on the central M-cholinoreceptors, resulting in characteristic tremors [1]. In view of this, I is widely used in the study of central M-cholinoreceptors.

In the plan of a further study of the active surface of cholinoreceptors, we synthesized certain cyclic and aliphatic analogs of I.

The presence of hydroxyl and acid portions of the molecule of amizil and its alkyl derivatives is a deciding factor for the enhancement of M-cholinolytic properties of these compounds [2]. Therefore we were also interested in determining how the activity of I changes when hydroxyl is introduced into the γ-position of the piperidine ring.

Cyclic analogs of I were produced according to the following schemes:



THe initial β,β'-dicarbomethoxydiethylmethylamine (II) was produced according to the methods described in the literature on the basis of acrylonitrile [3] or methyl acrylate [3-7]. In contrast to the literature data, the reaction of methyl acrylate with methylamine was conducted in aqueous solution; the yield of II was 63-68%. The sodium enolate of N-methyl-3-carbomethoxy-4-piperidone (III) was produced by cyclization of II in xylene solution in the presence of sodium methylate. N-Methyl-3-carbomethoxy-4-piperidone was isolated from the Na-enolate III by the method of [8]; it was converted to the hydrochloride (IV) by passage of dry hydrogen chloride into a toluene solution of it. Yield 80.8%, mp. 170-172°C [3].

To obtain a mixture of stereoisomers of N-methyl-3-carbomethoxy-4-hydroxypyridine (V) we used an electrochemical method of reduction in acid medium, proposed for the carboethoxy derivative [9].

The mixture of stereoisomers (V) in the form of the hydrobromide was separated into cis-(VI) and trans-(VII) forms by the method of [10]. In the interaction with methyl iodide, V gave the methiodide (VIII).

Catalytic reduction of I on skeletal nickel under atmospheric pressure yielded N-methyl-3-carbomethoxypiperidine (IX). The theoretical amount of hydrogen was absorbed, and the absorption band of C=C at 1665 cm^{-1} disappeared in the IR spectra, while the frequency of the ester group was shifted from 1725 cm^{-1} for I to 1745 cm^{-1} for IX.

Aliphatic analogs of I — methyl esters of dimethyl- and diethylaminoisobutyric acids — were synthesized by the reaction of methyl methacrylate with dimethyl- and diethylamine at room temperature [11]. Their hydrobromides X and XI were produced by the passage of dry hydrogen bromide into ether solutions of the corresponding bases.

Scientific-Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 2, pp. 180-184, February, 1983. Original article submitted May 12, 1982.

0091-150X/83/1702-0127$07.50 © 1983 Plenum Publishing Corporation

$(CH_3)_2NCH_2CH(CH_3)COOCH_3 \cdot HBr$
X
$(C_2H_5)_2NCH_2CH(CH_3)COOCH_3 \cdot HBr$
XI

EXPERIMENTAL CHEMICAL SECTION

β,β'-Dicarbomethoxydiethylmethylamine (II). To a mixture of 43.5 g sodium acetate, 55.2 g methylamine hydrochloride, and 100 ml of water, we added 141.2 g methyl acrylate. The mixture was mixed for 1 h and then heated on a water bath for 1 h at 80-85°C. It was cooled, the upper layer removed, and the aqueous layer extracted with benzene. After the removal of benzene the residue was redistilled under vacuum. Yield 114 g (68.4%) II, bp 140-142°C (18 mm), n_D^{20} 1.4450.

Na-Enolate of N-Methyl-3-carbomethoxy-4-piperidone (III). To a suspension of 62.3 g sodium methylate in 300 ml of dry xylene we added 212 g of freshly redistilled II. The temperature thereupon rose to 35°C. Then the mixture was heated on an oil bath at 140-150°C. As the methanol liberated was removed, the temperature in the flask was raised to 130°C. The reaction mixture was cooled, the Na-enolate filtered, and washed on the filter with xylene and ether. Yield 100%; when the reaction was conducted in toluene the yield was lowered to 65%.

Methiodide of N-Methyl-3-carbomethoxy-4-hydroxypyridine (VIII). A 5.32 g portion of N-methyl-3-carbomethoxy-4-hydroxypyridine (V) was dissolved in 10 ml of dry acetone, and 2 ml of methyl iodide was added with cooling. It was left for 5 h, the precipitate filtered off, washed with dry acetone, and dried in a vacuum desiccator. Yield 9.23 g (94.7%) VIII, mp 173-179°C (with dec.). It was recrystallized from absolute alcohol, mp 184-186°C. Found, %: I 40.58; N 4.43. $C_9H_{18}NIO_3$. Calculated, %: I 40.27, N 4.44.

N-Methyl-3-carbomethoxypiperidine (IX). In a flask for hydrogenation we placed 10.53 g of freshly redistilled I in 100 ml of ethanol, added about 2 g of skeletal nickel, and passed hydrogen through with shaking. The theoretical amount of hydrogen was absorbed in 65 min. The catalyst was filtered off, the alcohol distilled off, and the residue redistilled under vacuum. Yield 8.73 g (82.3%) IX, bp 79-80°C (10 mm), n_D^{20} 1.4558 [12]. Hydrobromide, mp 105-107°C (from a mixture of alcohol and ether) [13].

Hydrobromides of Dimethyl- and Diethylaminoisobutyric acids (X and XI) were obtained by passing the calculated amount of dry hydrogen bromide into an ether solution of the corresponding base with cooling with ice water. The precipitate was filtered off, washed with dry ether, and crystallized from a mixture of dry acetone and ether. X: mp 134-135°C. Found, %: Br 35.06; N 6.22. $C_7H_{16}BrNO_2$. Calculated, %: Br 35.33; N 6.19. XI: mp 95-96°C. Found, %: Br 31.61; N 5.59. $C_9H_{20}BrNO_2$. Calculated, %: Br 31.43; N 5.51.

EXPERIMENTAL PHARMACOLOGICAL SECTION

The experiments were conducted on male white mice weighing 18-23 g. The acute toxicity of the drugs and their ability to cause tremors and salivation were determined. The action of all the substances was studied in the dose range from 1 to 2000 mg/kg or more in the case of intraperitoneal injection. In the determination of toxicity, 10 animals were used for one dose of the drug. The results of the experiment were treated statistically according to the Kerber method. In seeking the doses at which the substance gives an M-cholinomimetic effect (tremors and salivation), 6-10 animals were taken for each dose of the drug. Statistical treatment of the experimental data was performed by the method of Miller and Tainter. The standard errors were calculated at $P = 0.05$.

When injected intraperitoneally in a dose of 12 mg/kg, I induced tremors in 100% of the animals. Salivation was observed in 100% of the animals at a dose of 15 mg/kg. The central and peripheral effects of I were blocked by metamizil in a dose of 1 mg/kg. The results of the pharmacological investigation of I and the new compounds are presented in Table 1, from which it is evident that hydrogenation of the ring of the molecule I leads to a sharp decrease in the toxicity of the preparation IX and in the m-cholinomimetic activity, since weak tremors were observed when it was used only at a dose of 800-1000 mg/kg, and salivation at 1500 mg/kg. Tremors and salivation were noted in 100% of the animals when IX was given in a dose of 2000 mg/kg, and they were blocked by metamizil in a dose of 1 mg/kg.

The introduction of a hydroxyl into the γ-position of the piperidine ring almost entirely deprives the compounds VI and VII obtained of M-cholinomimetic properties. In doses

TABLE 1. M-Cholinomimetic Activity of Derivatives of Piperidine and Dialkylaminoisobutyric Acids

Compound	LD_{50}, mg/kg	ED_{50}, mg/kg	
		tremors	salivation
I		6,15 (5,19—7,11)	11,3 (10,64—11,96)
IV	655	0	0
VI	2000	0	0
VII	2000	0	0
VIII	570	0	0
IX	2000	1100 (821,1—1378,9)	1100 (1293—1506,1)
X	800	440 (326,7—553,3)	300 (265—335)
XI	2000	0	0

Note. The limits of fluctuations are indicated in parentheses.

exceeding 2000 mg/kg, VI and VII induced only a certain decrease in the motor activity of the animals. However, for the quaternary salt VIII the toxicity was increased, and a weak peripheral M-cholinomimetic activity appeared. It is known that quaternary amines penetrate poorly through the blood-brain barrier; evidently for this reason compound VIII in a dose of 390 mg/kg induced only salivation, which was blocked by metamizil in a dose of 4 mg/kg. In a dose of 500 mg/kg, the action of VIII was characterized by the appearance of convulsions in addition to salivation. Since the convulsive dose of VIII is close to LD_{50}, and the convulsions were not blocked by the central M- and N-cholinolytics metamizil and eterophen, it can be assumed that in this case only the toxic action of the drug was manifested. Earlier [14], in an investigation of the M-cholinomimetic properties of derivatives of 5-ethoxy-1,2,5-trimethyl-4-piperidone, it was shown that replacement of the ethoxy group by hydroxyl leads to disappearance of the M-cholinomimetic activity.

The presence of a ketone group in the γ-position of the piperidine ring increases the toxicity of preparation IV in comparison with VI, VII, and IX. However, there are virtually no M-cholinomimetic properties for IV, since it did not induce salivation, and the tremors and convulsions arising when this drug was used in doses of 400-500 mg/kg were not blocked by metamizil and eterophen. No ability of IV to block nicotine convulsions were detected; however, there is a tendency to potentiate tremors and salivation induced by I. Therefore it can be assumed that IV has such a weak effect that it is not clearly manifested against a background of the toxic action of the preparation in large doses, and it is detected only in the ability to potentiate the action of I.

The methyl ester of X exhibits distinct M-cholinomimetic properties, since its action in doses of 300-600 mg/kg was accompanied by tremors and salivation. The tremors and salivation were blocked by metamizil in a dose of 5 mg/kg. The replacement of methyl radicals at the nitrogen by ethyl (XI) leads to the appearance of N-cholinolytic properties, since the tremors induced by this drug in a dose of 1500-2000 mg/kg were not blocked by metamizil in a dose of 5-10 mg/kg. However, preparation XI in doses of 1500-2000 mg/kg blocked nicotine convulsions.

Of the investigated compounds, only I itself exhibits pronounced M-cholinomimetic activity. This evidently depends to a substantial degree on the presence of a double bond in the piperidine ring of the molecule of I. It is precisely this bond that imparts a semirigid nature to the molecule, retaining it in a definite conformational state. The absence of a double bond substantially lowers the cholinomimetic activity. An analogous pattern was noted on the example of isoarecholine and arecaidine [15].

In a study of the influence of the hydroxyl group on certain chemical and biological properties of n-pentylammonium salts it was found that the hydroxyl group lowered the affinity of the compound for M-cholinoreceptors, since the activity of the synthesized substances is appreciably lowered in tests on guinea pig intestines [16].

Thus, an analysis of our own research results and the literature data indicates that a hydroxyl in the molecule of an M-cholinomimetic does not promote any increase in activity

in the interaction of the substance with the M-cholinoreceptor, but on the contrary, it is a deciding factor hindering such interaction. It can be assumed that hydroxyl in the cholinomimetic molecule interacts with some sort of supplementary active site of the cholinoreceptor, which hinders the complete realization of conformational rearrangements of the molecule of the substance and the molecule of the cholinoreceptor to a definite degree of compartmentalization necessary for interaction with the cholinoreceptor and the appearance of the cholinergic effect. It can be assumed that the hydroxyls in the molecule of the M-cholinomimetic and M-cholinolytic interact with the same active groups of the M-cholinoreceptor; in this case, under the action of M-cholinomimetic this bond reduces the activity, whereas the presence of hydroxyl in the acid portion of the molecule of central M-cholinolytics, close in chemical structure to acetylcholine, is a deciding factor for the interaction with the M-cholinoreceptor, manifested in an intensification of the M-cholinolytic properties of the drugs.

LITERATURE CITED

1. N. V. Golyakhovskii, Farmakol. Toksikol., 12, No. 1, 8 (1948).
2. N. A. Losev, "Pharmacological analysis of α- and β-derivatives of amizil and spasmolytin," Author's Abstract of Candidate's Dissertation [in Russian], Leningrad (1968).
3. N. A. Preobrazhenskii, K. M. Malkov, M. E. Maurit, et al., Zh. Obshch. Khim., 27, 3162 (1957).
4. L. E. Craig and D. S. Tarbell, J. Am. Chem. Soc., 71, 465 (1949).
5. R. Mozingo and J. McCracken, in: Syntheses of Organic Preparations [Russian translation], Collection 3, Moscow (1952), p. 178.
6. D. R. Hawtön, J. Org. Chem., 10, 277 (1945).
7. A. N. Cook and K. J. Reed, J. Chem. Soc., 399 (1945).
8. J. Levy and G. A. Bernotsky, Patent No. 2685583, 1954 (USA), Chem. Abstr., 49, 11024 (1955).
9. A. Dobrowsky, M. Chem., 83, 443 (1952).
10. M. E. Maurit and N. A. Preobrazhenskii, Zh. Obshch. Khim., 28, 968 (1958).
11. I. N. Nazarov and L. Z. Kazaryan, Zh. Obshch. Khim., 27, 3302 (1957).
12. N. Sperbel, M. Sherlock, D. Papa, et al., J. Am. Chem. Soc., 81, 704 (1959).
13. N. A. Preobrazhenskii and L. B. Fisher, Zh. Obshch. Khim., 11, 140 (1941).
14. N. A. Iskarev and K. S. Shadurskii, Farmakol. Toksikol., No. 2, 184 (1965).
15. L. Lambrecht and E. Mutschler, Experientia, 31, 1317 (1975).

INCORPORATION OF PROTEINS INTO ONE-LAYERED LIPOSOMES

A. I. Tentsova, Yu. Sh. Moshkovskii,
O. S. Zizina, E. A. Dolginova,
N. S. Kovaleva, and A. N. Rossel's

UDC 615.451.234:615.357.37].074

Liposomes have been attracting the attention of researchers for more than two decades. For a long time they were considered only as a convenient adequate model of natural biological membranes. Processes of diffusion of certain compounds through a lipid bilayer, ionic permeability of membranes of various phospholipid compositions [1, 2], the modeling of intercellular contact interactions [3], etc. can be studied with the aid of liposomes. In recent years the sphere of application of liposomes has expanded substantially in connection with the possibility of their use in medicine as carriers of drugs into the organism [4-6].

The creation of an effective liposomal form of a drug requires fundamental investigation of the physicochemical aspects of the interaction of the drug substance to be incorporated with the liposomes.

The purpose of the present work was to determine the mechanism of the binding of various

All-Union Scientific-Research Institute of Pharmacy, Moscow. Scientific-Research Institute for the Biological Testing of Chemical Compounds, Moscow Province.

Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 2, pp. 184-187, February, 1983. Original article submitted April 26, 1982.

0091-150X/83/1702-0130$07.50

© 1983 Plenum Publishing Corporation