EFFECT OF SOME PHENOTHIAZINE AND DIBENZAZEPIME DERIVATIVES ON THE MUSCARINIC CHOLINERGIC SYSTEM

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Replacement of the dialkylaminoalkyl (DAL) group (Fig. 1a) attached to the cyclic nitrogen atom in position 10 of the phenothiazine (PT) heterocycle and related tricyclic compounds (dibenzazepines – DBA) by a dialkylaminoacyl (DAC) group (Fig. 1b) leads to a significant change in the properties of these compounds [3].

The psychotropic properties of the DAC-derivatives are much weaker, but their cardiotropic activity is potentiated [3].

To discover the mechanisms responsible for differences in the action of different types of PD derivatives investigations confirming previous conclusions have been conducted on the molecular level [1]. It has been shown that in DAC-derivatives of tricyclic nitrogen-containing systems (PT, DBA), compared with their DAL-analogs, the basic mechanisms responsible for realization of psychotropic properties (binding with brain D2-receptors for PT; reduction of serotonin reuptake, and weakening with specific binding sites of imipramine for DBA) are significantly weakened [2].

The aim of this investigation was to compare the effects of DAL- and DAC-derivatives of PT and DBA (chlorpromazine, trifluoperazine, fluophenazine, imipramine, and chlorimipramine) and newly synthesized DAC-analogs (G-512. G-219 G-229. BI-5 and BI-3 respectively), and also of nonachlazine (NC) on muscarinic receptors (MR) of the rabbit striatum and heart and the rat brain.

EXPERIMENTAL METHOD

The classical MR antagonist [^{3}H]-quinuclidinyl benzylate (QNB) was used as radioligand in the substitution reactions [4, 10] The nonselectivity of QNB relative to the different subclasses of MR enables it to be widely used to study MR of the brain (M_1R, M_2R), heart (M_2R), and other organs and tissues [8, 14]. Isolation of plasma membranes from the rabbit striatum and from the rat brain, after removal of the cerebellum and brain stem, and also the rabbit heart, was carried out by the method in [8] with modifications. These brain regions contain up to 70-80% of M_1R [13, 14]. Binding of [^{3}H]-QNB with MR was measured by the method in [8] with modifications. The sample contained 50-200 μg protein. The concentration of [^{3}H]-QNB was $4.5 \cdot 10^{-10}$ M and its K_d was $8 \cdot 10^{-11}$ M. Protein was determined as in [11]. The following reagents were used: chlorpromazine, fluophenazine, and trifluoperazine as well as their DAC analogs (G-512, G-229, and G-219 respectively), were synthesized at the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, by Senior Scientific Assistant A. N. Gritsenko, and NC by Senior Scientific Assistant A. M. Likhosherstov. Imipramine and chlorimipramine were obtained from Sigma (USA); and their DAL-analogs BI-2 and BI-3 were synthesized by A. N. Gritsenko. Tris, imidazole, BSA, atropine, and Folin's reagent were obtained from Sigma (USA); [^{3}H]-QNB was obtained from Amersham (England); the NaH_2PO_4 and $MgSO_4{}^4$ was from Merck (Germany); the GF/C filters from Whatman (England).

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TABLE 1. LC_{50} (μM) for Displacement of Radioligand [^{3}H]-QNB from Muscarinic Receptors of Various Tissues by DAC- and DAL-Derivatives of PT and DBA

Compound	Tissue		
	rabbit striatum	rat brain (without cerebellum and brain stem)	rabbit heart
Trifluoperazine	8,0±0,07	—	42,2±4,3
G-219	3,5±0,03*	—	30,4±3,8
Fluorophenazine	8,5±0,08	—	40,7±4,0
G-229	10,2±1,1	—	51,2±4,9
Chlorphomazine	0,09±0,01	—	2,5±0,2
G-512	0,025±0,002*	—	0,32±0,04*
Nonachlazine	3,5±0,3	—	33,9±0,04
Imipramine	—	0,25±0,03	3,6±0,42
BI-1	—	0,19±0,022	2,7±0,30
Chlorimipramine	—	0,24±0,02	3,5±0,33
BI-3	—	0,13±0,01*	—

Legend. Mean values of 3 or 4 experiments are shown. ——) No data available. *$p < 0.05$) Significant differences between affinity of DAC-derivatives and DAL-analogs for MR.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the DAL-derivatives of PT and DBA, in micromolar concentrations, displaced [^{3}H]-QNB more or less actively from the central MR of the rabbit striatum and the rat brain, in agreement with the results described in [8]. The most active antimuscarinic agents were chlorpromazine, imipramine, and chlorimipramine The corresponding DAC-analogs as a rule had rather higher affinity for MR (Table 1). For instance, compounds G-219, BI-2, and BI-3, which are DAC-analogs of trifluoperazine, imipramine, and chlorimipramine respectively, had LC_{50} values 20-50% lower than the corresponding LC_{50} values of the DAL-agents. The DAC analog of chlorpromazine, preparation G-512, displaced [^{3}H]-QNB most actively from MR of the rabbit striatum, its LC_{50} being 0.025 μM. Preparation G-229, on the other hand, had lower affinity for MR (LC_{50} was about 20% higher than that of the corresponding DAL-analog, fluophenazine).

The antimuscarinic activity of some DAC-derivatives of PT and DBA may play a role in the realization of their cardiotropic action. Central muscarinic mechanisms control mainly the stimulating action of cholinergic agents on the heart rate and blood pressure [5, 15]. This takes place both through activation of central sympathetic neurons, leading to stimulation of peripheral sympathetic nerve endings, and through modulation of baroreflexes, and also through release of catecholamines from the adrenals [5, 15].

The M_2-blocking activity of all the compounds tested was much weaker against the rabbit heart (Table 1). The character of the action and basic principles were the same as those for MR of the brain. We know that the activity of many antiarrhythmics of the class of I-blockers of Na-channels correlates with their ability to bind with UR It has been suggested that there is allosteric interaction between the MR system and Na-channels [7]. The peripheral effects of M-agonists also are linked with an increase in strength of the cardiac contractions through stimulation of Na—Ca metabolism during depolarization and increased permeability of the sarcolemma for Na ions [9]. Activation of M_2-receptors at the cell level is usually associated with inhibition of adenylate cyclase, whereas regulation of phosphoinositide metabolism is effected through M_1-receptors [4]. There is also evidence that M_1R are involved in the mediation of inhibition of adenylate cyclase by acetylcholine and of the influence of M_2R on phosphoinositide metabolism [6].

Analysis of the curve of saturation of rabbit heart M_2R by the radioligand [^{3}H]-QNB in the presence of compound G-512 by Scatchard plot showed that inhibition of binding of [^{3}H]-QNB is competitive, with an inhibition constant K_i of $2.9 \cdot 10^{-8}$ M and a dissociation constant K_d of $9.5 \cdot 10^{-10}$ M (Figs. 1 and 2).

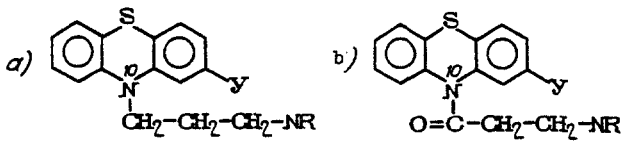

Fig. 1. Chemical structure of dialkylaminoalkyl (a) and dialkylaminoacyl (b) derivatives of phenothiazine.

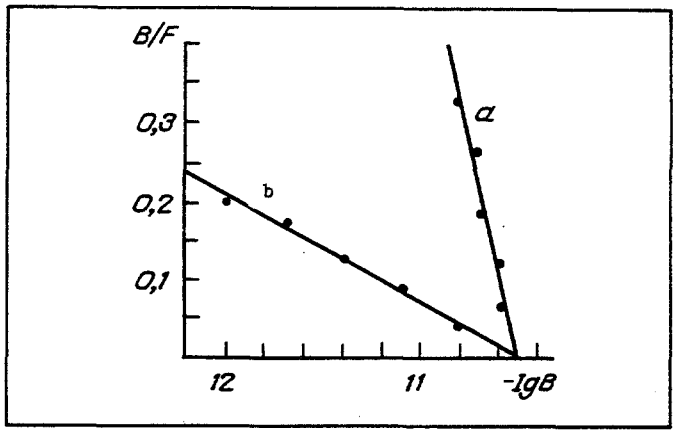

Fig. 2. Effect of compound G-512 on binding of [^{3}H]-QNB by M_2-receptors of ventricles of the rabbit heart (Scatchard plot). B) Concentration of [^{3}H]-QNB bound with MR, F) free concentration of [^{3}H]-QNB. a) In absence, b) in presence of G-512 ($3.2 \cdot 10^{-7}$ M).

Nonachlazine (NC), a preparation with antiischemic action, was found to have a moderate effect on the cholinergic M-system As our results show (Table 1), MC possesses moderate central (LC_{50} = 3.5 μM) and weak peripheral (LC_{50} = 33 μM) antimuscarinic activity. Considering the high therapeutic concentrations of MC, it can be tentatively suggested that a definite role in the mechanism of action of this preparation may be played by its central antimuscarinic activity.

Analysis of the curves of displacement of [^{3}H]-QNB from MR of plasma membranes of the rat brain and rabbit striatum and heart thus shows that several DAL- and DAC-derivatives of PT and DBA possess marked antimuscarinic activity in micromolar concentrations. In some cases DAC-derivatives have a rather stronger action on the cholinergic M-system than the corresponding DAL-analogs. Compound G-512, the DAC-derivative of chlorpromazine, was found to have the greatest affinity for MR. It was shown on the example of G-512 that inhibition of binding of [^{3}H]-QNB with MP in the presence of PT derivatives is competitive in character. A definite role in the mechanism of realization of the pharmacologic action of the antianginal preparation NC may be played by its central antimuscarinic activity.

On the example of affinity for MR, the presence and similarity of effects of the DAC- and DAL-derivatives of two tricyclic nitrogen-containing systems is confirmed.

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