Biochemical Pharmacology, Vol.29, pp.1311-1314.
Pergamon Press Ltd. 1980. Printed in Great Britain.

SIMILARITIES IN THE BINDING SITES OF THE MUSCARINIC RECEPTOR AND THE IONIC CHANNEL OF THE NICOTINIC RECEPTOR

Robert S. Aronstam, Amira T. Eldefrawi and Mohyee E. Eldefrawi

Department of Pharmacology and Experimental Therapeutics,

University of Maryland School of Medicine, Baltimore, Maryland 21201

(Received 3 December 1979; accepted 25 January 1980)

The nicotinic acetylcholine (ACh) receptor is identified by its specific binding of ACh and α-bungarotoxin (1), while muscarinic ACh-receptors are identified by their specific binding of quinuclidinyl benzilate (QNB) (2). The ionic channel of the nicotinic receptor has sites which are pharmacologically distinct from the nicotinic receptor sites, since they bind specifically perhydrohistrionicotoxin (H_{12}-HTX) and amantadine (3,4). Although nicotinic and muscarinic receptor sites differ widely in their drug specificities, electrophysiological studies reveal that several muscarinic antagonists interact with sites on the ionic channel of the nicotinic receptor, thereby altering the time course of endplate currents in a voltage-dependent manner (5). In addition, phencyclidine (PCP) and phencyclidine methiodide (PCP-MeI) act as antagonists of muscarinic receptor sites (6) and also interact with sites on the ionic channel (7). The present study was initiated to probe into the extent of similarities between muscarinic receptor and nicotinic ionic channel binding sites.

Materials and Methods. Chemicals. L-Quinuclidinyl [phenyl 4(n)-^{3}H]benzilate ([^{3}H]QNB, 44 Ci/mmol) was from Amersham, and the unlabeled and tritiated H_{12}-HTX (21 Ci/mmole) were provided by Dr. John Daly of NIH. Adiphenine, PCP and PCP-MeI, (2S, 4S) and (2R, 4R) 2,2-diphenyl-4-(2-dimethylaminoethyl)-1,3-dioxolane methiodides, trans-2-methyl-4-(2-dimethylaminoethyl)-1,3-dioxolane methiodide and RS(±), R(+) and S(-) hyoscyamine were from Dr. David J. Triggle of the State University of New York. Other drugs used were dibucaine-HCl (CIBA-Geigy), tetracaine-HCl and procaine-HCl (Schwarz/Mann), quinacrine-HCl and amantadine-HCl (Sigma), pilocarpine-HCl and scopolamine-HBr (Polysciences), tetraethylammonium bromide (Baker), lidocaine (Astra), piperocaine (Lilly), prilocaine and dimethisoquin (Laboratoire Roger Bellon).

Binding assays. Membranes were prepared from the cerebral cortex of Wistar rats for muscarinic receptor studies and from the electric organ of Torpedo ocellata for nicotinic channel studies and the binding measured by filtration assays as previously described (7,8). When measuring muscarinic binding, the protein content was 50 μg/ml, the [^{3}H]QNB concentration 100 pM, and the receptor concentration 20 pM. Nonspecific binding was determined by running a duplicate series of incubations containing 5 μM scopolamine and was always less than 5% of the total binding. When measuring nicotinic ion channel binding, the microsac membrane protein was 25-50 μg/ml and the [^{3}H]H_{12}-HTX concentration 2 nM. Nonspecific binding was determined by including 5 mM amantadine-HCl in a duplicate incubation series and represented less than 4% of the total binding. Ion channel binding was measured in the absence and presence of 1 μM carbamylcholine (carb). In both assays the buffer was 50 mM Tris-HCl, pH 7.4, the total incubation volume 1 ml and the time of incubation 2 h. Drug concentrations that inhibit 50% of the binding (IC_{50}) were determined by including the unlabeled drugs at 5 to 8 concentrations in otherwise identical incubation media. The inhibition constant (K_i) was calculated according to the relationship, $K_i = IC_{50}/(1+[F]/K_D)$, where [F] is concentration of free radiolabeled ligand, and K_D is its dissociation constant from the binding site. In the case of [^{3}H]H_{12}-HTX, the ratio $[F]/K_D$ was 0.034 and 0.012 in presence and absence of 1 μM carb, respectively. For [^{3}H]-QNB binding $[F]/K_D$ was close to 1.0.

Results. All the 23 drugs tested inhibited competitively the specific binding of [^{3}H]QNB to the muscarinic ACh-receptor sites in membranes from rat brain cortex and [^{3}H]H_{12}-HTX to sites on the ionic channel associated with the nicotinic ACh-receptor in membranes from Torpedo electroplax. This is illustrated for representative compounds in Fig. 1. Muscarinic antagonists inhibited [^{3}H]QNB binding with K_i values in the range of 0.08-10 nM, while the K_i's for agonists (pilocarpine and methyldioxolane) were in the μM range (Table 1). The muscarinic drugs were much less potent in inhibiting [^{3}H]H_{12}-HTX binding as evidenced by the K_i values in the μM to mM range and the lack of any inhibition by pilocarpine or methyldioxolane even at 1 mM.

All the local anesthetics tested inhibited [^{3}H]QNB binding to muscarinic receptors with

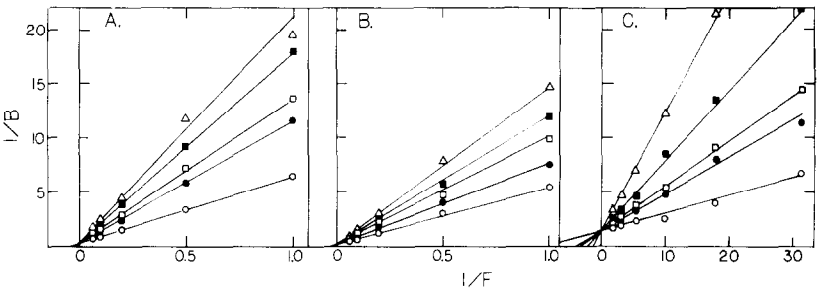

Fig. 1. Double reciprocal plots of the specific binding of [^{3}H]H_{12}-HTX to ionic channels of nicotinic receptors from Torpedo electroplax in absence (A) and presence of carb (B), and of [^{3}H]QNB to muscarinic receptors from rat brain cortex (C). B, concentration of bound [^{3}H]H_{12}-HTX in pmoles/mg protein or [^{3}H]QNB expressed as a fraction of the maximal binding of 1.34 pmoles/mg protein; F, concentration of free radiolabeled ligand in nM. In 1A, binding was in presence of 30 μM tetracaine (●), 10 μM PCP (□), 5 μM dimethisoquin (■) or 10 μM dibucaine (Δ) or in absence of competing drug (o). In 1B, binding was in presence of 20 μM tetracaine (●), 2 μM PCP (□), 0.4 μM dimethisoquin (■) or 0.5 μM dibucaine (Δ) or in absence of competing drug (o). In 1C, binding was in presence of 30 μM dibucaine (●), 2 μM dimethisoquin (□), 20 μM tetracaine (■) or 1 μM quinacrine (Δ) or in absence of competing drug (o).

K_i values in the 2 μM to 640 μM range. Except for piperocaine and dimethisoquin, they were less potent in this regard than any of the classical muscarinic drugs. As for their effects on [^{3}H]H_{12}-HTX binding, lidocaine and prilocaine did not inhibit binding at 1 mM, and procaine was a poor inhibitor, while dibucaine, dimethisoquin, piperocaine and tetracaine were relatively strong. Whereas piperocaine and tetracaine were much more potent in inhibiting [^{3}H]H_{12}-HTX binding than [^{3}H]QNB binding, prilocaine, lidocaine and procaine had the reverse potencies. Among the other ionic channel drugs and toxins, H_{12}-HTX was the poorest inhibitor of [^{3}H]QNB binding with a K_i of 1.6 mM, while quinacrine and PCP-MeI were the most potent with K_i values of 0.4 μM and 0.8 μM, respectively. The K_i values on [^{3}H]H_{12}-HTX binding for drugs belonging to this group were in the 0.1-10 μM range.

It was previously demonstrated that the affinity of several compounds, including piperocaine (9) and H_{12}-HTX (10), for the ionic channel was altered by receptor activation. The affinity of [^{3}H]H_{12}-HTX for the ionic channel was at least doubled in presence of 1 μM carb (Table 1), but since the concentration of [^{3}H]H_{12}-HTX used (2 nM) was so low compared to its K_D value (≈100 nM), differences in the IC_{50} values in the presence and absence of carb accurately reflected changes in the affinity of the competing ligand independent of the change in [^{3}H]H_{12}-HTX binding. The selectivity of certain drugs for the muscarinic vs the ionic channel sites depended on whether or not the ion channel affinity was measured in presence of carb. Thus, while in the absence of carb prilocaine and dimethisoquin inhibited [^{3}H]QNB binding more strongly than [^{3}H]H_{12}-HTX binding, the opposite was true for tetraethylammonium (TEA) and tetracaine, and these specificities were reversed in the presence of carb. Carb lowered the K_i of ionic channel sites for PCP, PCP-MeI, quinacrine, adiphenine, QNB, dimethisoquin, dibucaine, lidocaine, prilocaine and H_{12}-HTX, raised the K_i of piperocaine, tetracaine, amantadine and TEA and had little effect on procaine. Carb was not the only receptor activator to affect [^{3}H]H_{12}-HTX binding to ionic channel sites; in fact ACh (0.1 μM) and nicotine (1 μM) had the same qualitative effects.

The negative log of the K_i's for drug interactions with the two sites are compared in Fig. 2. Scopolamine, the atropine and diphenyldioxolane enantiomers and QNB had up to 100,000-fold higher affinity for muscarinic ACh-receptors compared to nicotinic ion channels, while H_{12}-HTX had at least a 10,000-fold selectivity in the opposite direction. Even when H_{12}-HTX and the muscarinic drugs were excluded from the calculations, a weak correlation was obtained in drug affinities for the muscarinic ACh-receptor sites and the ionic channel sites. Linear regression analysis gave a slope of 0.45, a Y intercept of 2.7 and a correlation coefficient of 0.48. The correlation was greatly improved by the inclusion of 1 μM carb when measuring affinities to the ionic channel sites, which brought the affinities of PCP, PCP-MeI, quinacrine, adiphenine, piperocaine, tetracaine, prilocaine and lidocaine closer to the line of identity between muscarinic and ion channel sites. A slope of 0.81, a Y intercept of 0.99 and a correlation coefficient of 0.75 were then obtained. These changes were due to decreased affinity of certain compounds (piperocaine and tetracaine) and increased affinity of the others. On the other hand, nicotinic receptor activation by carb increased the difference between muscarinic and ionic channel activity of dimethisoquin, dibucaine, amantadine and TEA.

Discussion. A number of anticholinergic drugs (e.g. QNB, TEA, scopolamine, atropine, adiphenine), local anesthetics (e.g. procaine, tetracaine, lidocaine, dimethisoquin, piperocaine, prilocaine) and chemotherapeutic drugs (e.g. amantadine, quinacrine) inhibit competitively (Fig. 1) the binding of ligands to both muscarinic ACh-receptors of rat brain and ionic channel sites of the nicotinic receptor of Torpedo electric organs, but with widely varying selectivities (Table 1, Fig. 2). Certain compounds (e.g. H_{12}-HTX and muscarinic drugs) have

Table 1. Inhibition of [^{3}H]QNB binding to muscarinic ACh-receptor sites in rat brain and [^{3}H]H_{12}-HTX binding to ionic channel sites of the nicotinic ACh-receptor in Torpedo electroplax in the absence (control) and presence of 1 μM carb

Drugs	Inhibition constants (K_i)[a]		
	Muscarinic receptor	Ionic channel	
		Control	Carbamylcholine
I. Muscarinic:			
1. Diphenyldioxolane[b]	2.1 ± 0.2 x 10^{-9}[c]	1.5 ± 0.1 x 10^{-5}	2.5 ± 0.6 x 10^{-6}
2. Diphenyldioxolane[d]	3.5 ± 0.4 x 10^{-9}[c]	7.7 ± 2.1 x 10^{-6}	3.9 ± 0.8 x 10^{-6}
3. RS(±) hyoscyamine	5.4 ± 0.2 x 10^{-10}[c]	7.5 ± 1.3 x 10^{-4}	9.0 ± 1.8 x 10^{-4}
4. R(+) hyoscyamine	1.0 ± 0.1 x 10^{-8}[c]	1.0 ± 0.8 x 10^{-3}	6.2 ± 3.1 x 10^{-4}
5. S(-) hyoscyamine	3.2 ± 0.1 x 10^{-10}[c]	8.9 ± 1.7 x 10^{-4}	7.3 ± 0.9 x 10^{-4}
6. Methyldioxolane[e]	5.5 ± 0.2 x 10^{-6}[c]	>1 x 10^{-3}	>1 x 10^{-3}
7. Pilocarpine	1.7 ± 0.1 x 10^{-6}	>1 x 10^{-3}	>1 x 10^{-3}
8. QNB	8.2 ± 0.8 x 10^{-11}	1.5 ± 0.1 x 10^{-4}	8.3 ± 0.1 x 10^{-5}
9. Scopolamine	8.3 ± 1.1 x 10^{-10}	2.7 ± 0.4 x 10^{-3}	1.3 ± 0.2 x 10^{-3}
II. Ionic channel:			
A. Local Anesthetic			
10. Dibucaine	3.1 ± 1.2 x 10^{-5}	1.1 ± 0.6 x 10^{-5}	5.0 ± 0.1 x 10^{-7}
11. Dimethisoquin	2.0 ± 1.0 x 10^{-6}	4.8 ± 1.2 x 10^{-6}	3.8 ± 0.7 x 10^{-7}
12. Lidocaine	2.3 ± 0.3 x 10^{-4}	>1 x 10^{-3}	1.5 ± 0.1 x 10^{-4}
13. Piperocaine	6.0 ± 1.0 x 10^{-6}	3.2 ± 0.8 x 10^{-7}	4.2 ± 1.1 x 10^{-6}
14. Prilocaine	6.4 ± 0.7 x 10^{-4}	>1 x 10^{-3}	9.5 ± 2.2 x 10^{-5}
15. Procaine	1.4 ± 0.6 x 10^{-5}	7.9 ± 0.8 x 10^{-4}	6.9 ± 1.2 x 10^{-4}
16. Tetracaine	2.0 ± 1.2 x 10^{-5}	3.0 ± 0.1 x 10^{-6}	2.0 ± 0.2 x 10^{-5}
B. Other			
17. Adiphenine	1.2 ± 0.3 x 10^{-6}	2.8 ± 0.8 x 10^{-6}	2.1 ± 0.1 x 10^{-6}
18. Amantadine	2.7 ± 0.4 x 10^{-4}	4.0 ± 0.6 x 10^{-5}	2.0 ± 0.2 x 10^{-4}
19. H_{12}-HTX	1.6 ± 1.2 x 10^{-3}	1.1 ± 0.1 x 10^{-7}	8.2 ± 1.4 x 10^{-8}
20. PCP	1.5 ± 0.3 x 10^{-6}	1.0 ± 0.4 x 10^{-5}	2.2 ± 0.6 x 10^{-6}
21. PCP-MeI	4.0 ± 0.1 x 10^{-7}	1.1 ± 0.5 x 10^{-5}	2.0 ± 0.3 x 10^{-6}
22. Quinacrine	8.2 ± 0.3 x 10^{-7}	1.9 ± 0.1 x 10^{-6}	8.9 ± 0.2 x 10^{-7}
23. TEA	2.6 ± 0.9 x 10^{-4}	9.5 ± 2.1 x 10^{-5}	6.1 ± 0.4 x 10^{-4}

[a]The values listed in M represent the mean ± standard deviation for 3 separate determinations.
[b](2S,4S)2,2-diphenyl-4-(2-dimethylaminoethyl)-1,3-dioxolane methiodide.
[c]From ref. 8.
[d](2R,4R)2,2-diphenyl-4-(2-dimethylaminoethyl)-1,3-dioxolane methiodide.
[e]Trans-2-methyl-4-(2-dimethylaminoethyl)-1,3-dioxolane methiodide.

very high affinity for the ionic channel and muscarinic receptor sites, respectively, but the majority of local anesthetics and identified channel drugs affect each site with K_i values in the range from 0.1 to 10 μM. Muscarinic receptor binding sites have well defined structural geometries, which are reflected in the stereoselective binding of the hyoscyamine enantiomers (8). However, these isomers bind to ionic channel sites with identical weak affinities (Table 1). Correlations of the affinities of muscarinic receptor sites with the sites on the nonactivated (closed) ionic channels measured in vitro are weak with a correlation coefficient of 0.48 even after excluding H_{12}-HTX and muscarinic drugs (Fig. 2).

H_{12}-HTX stands out among the ionic channel drugs because of its relatively high affinity for these sites, combined with a very low affinity for the muscarinic receptor sites (Table 1, Fig. 2). In contrast, all the muscarinic drugs tested biochemically, except for diphenyldioxolane, have low affinities for the ionic channel sites. Nonetheless, electrophysiological investigations reveal ionic channel effects by muscarinic antagonists (e.g. atropine and scopolamine (5)) in the μM range. There are several possible explanations for these few discrepancies between biochemical and biophysical data. The effects of muscarinic drugs on the amplitude and time course of miniature endplate currents are voltage dependent (5), while the nature and extent of the membrane potential in the microsacs are unknown. It is quite possible that the ionic channel of the nicotinic receptor has several drug binding sites, and H_{12}-HTX and muscarinic drugs bind to different sites with high affinities, i.e. H_{12}-HTX does not identify all the physiologically relevant sites of drug interaction with the ionic channel. It is also possible that there are tissue differences in the cholinergic receptor-channel complex between Torpedo electroplax and frog muscles which were used in the electrophysiological studies.

The kinetics of [^{3}H]H_{12}-HTX binding to the ionic channel have led to suggestions of the presence of diffusional barriers restricting access of the toxin to channels in the resting conformation, barriers which are diminished greatly by opening of the channels by receptor agonists (10). With muscarinic drugs, the presence of carb either has no effect or slightly increases (maximum of 2-fold) their affinities for the ionic channel sites (Table 1), except for diphenyldioxolane where the K_i value decreases almost 5-fold. As for the ionic channel drugs, the presence of carb has different effects on the various drugs, decreasing the affinities of three and increasing the affinities of the majority. Nevertheless, receptor activation by carb improves the correlation between the affinities of the drugs for ionic channel and muscarinic receptor sites, increasing it from 0.48 to 0.75 (Fig. 2).

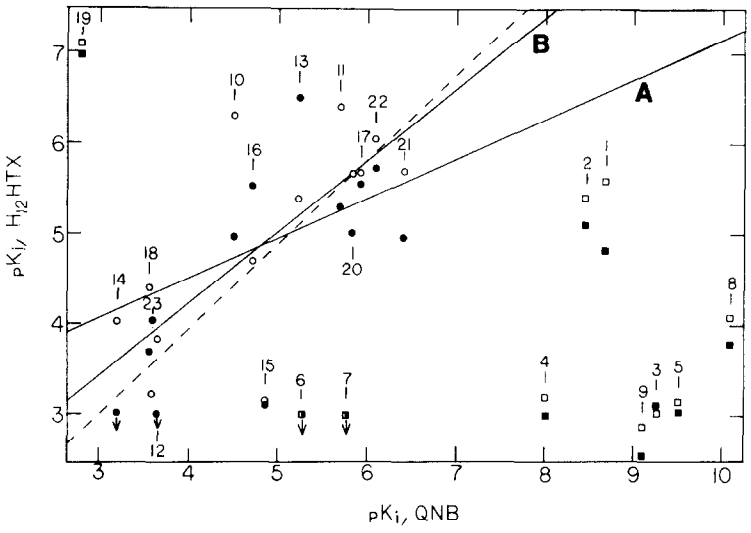

Fig. 2. Comparison of drug affinities for the ionic channel of the nicotinic ACh-receptor and the muscarinic ACh-receptor. The negative logarithms of the inhibition constants (pK_i) of [^{3}H]H_{12}-HTX binding to the ionic channel are plotted as a function of pK_i's for inhibition of [^{3}H]QNB binding to the muscarinic receptor. Ionic channel affinity was measured in the absenc (closed figures) and presence (open figures) of 1 μM carb. Drug numbers are identified in Table 1. The dashed line is the line of identity for the relationship pK_i, muscarinic recepto = pK_i, ionic channel. Line A describes the relationship between ionic channel affinity measured in the absence of a receptor activator and muscarinic affinity as revealed by linear regression analysis (r = 0.48, m = 0.45). Line B describes the corresponding relationship for the case in which ionic channel affinity was measured in the presence of 1 μM carb (r = 0.75, m = 0.81). Arrows below four symbols indicate that their pK_i values are below 3. Only the drugs represented by circles were included in the linear regression analyses.

There are many similarities between the muscarinic receptors and nicotinic ionic channels such as in their pharmacological specificities (Fig. 2, Table 1), in their possession of SH groups alkylation of which inhibits ligand binding, and in their reaction to changes in pH, temperature and ionic composition of the binding media (11, also unpublished). However, the many differences in their binding sites revealed in the present study argue against a close molecular relationship.

ACKNOWLEDGMENTS

We are most grateful to Drs. J. W. Daly and D. J. Triggle for kindly providing us with [^{3}H]H_{12}-HTX and several drugs, respectively, and to Dr. E. X. Albuquerque for his discussions and valuable criticisms. This research was supported by NSF grant BNS76-21683, NIH grant NS-15261 and Army Research Office grant No. DAAG 29-78-G-0203.

REFERENCES

1. M.E. Eldefrawi and A.T. Eldefrawi, In Advances in Cytopharmacology (B. Ceccarelli and F. Clementi, eds.) 3, 213, Raven Press, New York (1979).
2. S.H. Snyder, K.J. Chang, M.J. Kuhar and H.I. Yamamura, Fed. Proc. 34, 1915 (1975).
3. M.E. Eldefrawi, A.T. Eldefrawi, N.A. Mansour, J.W. Daly, B. Witkop and E.X. Albuquerque, Biochemistry 17, 5474 (1978).
4. M.-C. Tsai, M.A. Mansour, A.T. Eldefrawi, M.E. Eldefrawi and E.X. Albuquerque, Mol. Pharmacol. 14, 787 (1978).
5. M. Adler and E.X. Albuquerque, J. Pharmacol. Exp. Ther. 196, 360 (1976).
6. K. Jim, D.J. Triggle, E.X. Albuquerque, R.S. Aronstam and M.E. Eldefrawi, Fed. Proc. 38, 274 (1979).
7. E.X. Albuquerque, M.-C. Tsai, R.S. Aronstam, B. Witkop, A.T. Eldefrawi and M.E. Eldefrawi Proc. Nat. Acad. Sci. USA (in press).
8. R.S. Aronstam, D.J. Triggle and M.E. Eldefrawi, Mol. Pharmacol. 15, 227 (1979).
9. T.N. Tiedt, E.X. Albuquerque, N.M. Bakry, M.E. Eldefrawi and A.T. Eldefrawi, Mol. Pharmacol. 16, 909 (1979).
10. M.E. Eldefrawi, R.S. Aronstam, N.M. Bakry, A.T. Eldefrawi and E.X. Albuquerque, Proc. Nat. Acad. Sci. USA (in press).
11. R.S. Aronstam, L.G. Abood and J. Baumgold, Biochem. Pharmacol. 26, 1689 (1977).