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Antagonism of the five cloned human muscarinic cholinergic receptors expressed in CHO-K1 cells by antidepressants and antihistaminics

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Abstract—Based on molecular cloning studies, five different muscarinic receptor subtypes exist: m1, m2, m3, m4, and m5. We determined the affinity and selectivity of binding for sixteen antidepressants, two of their metabolites, and three antihistaminics (H_1) at these subtypes. Using Chinese hamster ovary cells (CHO-K1) transfected with genes for the human muscarinic receptor subtypes, we obtained equilibrium dissociation constants (K_ds) from competitive radioligand binding studies with [^{3}H]-quinuclidinyl benzilate ([^{3}H]QNB) and membranal preparations of these cells. QNB was the most potent compound studied (K_d 30–80 pM). Mequitazine (K_d 6–14 nM) and amitriptyline (K_d 7–16 nM) exhibited the highest affinity among the antihistaminics and antidepressants, respectively. Among the antidepressants examined were the serotonin-selective drugs sertraline and fluoxetine, both of which displayed K_d values >1 μM. The remaining antidepressants were moderate to weak antagonists with some eliciting no radioligand competition at high concentrations. The compounds studied showed no significant selectivity among the five cloned subtypes.

Antidepressants are known antagonists at the muscarinic receptor. The ability of a drug to block receptor binding can be related to its potential to cause undesirable side effects. Those side effects associated with muscarinic receptor blockade include dry mouth, blurred vision, constipation, urinary retention, sinus tachycardia, and memory dysfunction [1, 2]. Knowledge of the equilibrium dissociation constants (K_ds) for a series of antidepressants would help a clinician in the choice of a drug. Many investigators over the years have obtained such data. However, since the earlier studies, new antidepressants have been developed and, more important, molecular biologists showed that there exist five subtypes of the muscarinic receptor [3, 4]. With five different Chinese hamster ovary (CHO-K1*) cell lines stably expressing the five different human muscarinic receptors [3, 4] we determined the K_d values of sixteen antidepressants, two of their metabolites, and three antihistaminics at these five receptor subtypes. Here we present the results of these studies.

Materials and Methods

Sources of materials. The CHO-K1 cell lines were gifts from Dr. T. Bonner (National Institute of Mental Health, Bethesda, MD). The radiochemical used was [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) (32.9 Ci/mmol; Dupont New England Nuclear, Boston, MA). Manufacturers provided the following compounds: mequitazine (Pharmuka Lab, France); cyproheptadine (Merck Sharp & Dohme Research Laboratory, West Point, PA); adinazolam mesylate (Upjohn Co., Kalamazoo, MI); bupropion HCl (Burroughs Wellcome Co., Research Triangle Park, NC); dothiepin (Marion Lab Inc., Kansas City, MO); doxepin HCl (Pfizer, Inc., Brooklyn, NY); etoperidone HCl (Angelini Pharmaceuticals, Riveredge, NJ); femoxetine HCl (Novo Nordisk, Målov, Denmark); fluoxetine, norfluoxetine maleate, and nortriptyline HCl (Eli Lilly and Co., Indianapolis, IN); lofepramine HCl (Kabi Pharmacia Therapeutics, Helsingborg, Sweden); paroxetine HCl (SmithKline Beecham Pharmaceuticals, Surrey, U.K.); sertraline and desmethylsertraline (Pfizer Central Research, Inc., Groton, CT); trazodone (Mead Johnson Co., Evansville, IN); and venlafaxine (Wyeth-Ayerst, Princeton, NJ). Orphenadrine, amitriptyline HCl, desipramine HCl, and imipramine were obtained from the Sigma Chemical Co. (St. Louis, MO).

Cell culture and membranal preparation. CHO-K1 cells (passage numbers 7–17) were plated in 150 cm^2 petri dishes. We have confirmed by northern blot analysis that the clones expressed the appropriate human muscarinic receptor subtype [5]. Cells were grown and passaged as previously described [5]. Membranes from confluent CHO-K1 cells were prepared as described [5]. Preparations were frozen in liquid nitrogen for storage until an adequate amount was accumulated for use in an assay. The method of Lowry *et al.* [6] was used to determine the protein content. Bovine serum albumin was used as the standard.

Radioligand binding assays. Assays were performed on the Beckman Biomek 1000, Automated Laboratory Workstation [7]. The amount of membrane protein added to the assay tube varied inversely with the level of receptor expression in each subtype cell line (m1 = 7 μg, m2 = 100 μg, m3 = 15 μg, m4 = 40 μg, and m5 = 125 μg). Total assay volume was 1.0 mL containing 0.03 nM [^{3}H]-QNB and various concentrations of antidepressants or antihistamines as previously described [5]. The difference between binding (zero unlabeled ligand) and nonspecific binding (excess unlabeled ligand) determined the specific [^{3}H]QNB binding to the muscarinic receptor. Specific binding of added ligand represented 10–40% of total amount added, while nonspecific binding was less than 10% of the total binding.

Analysis of data. We analyzed the data by using the LIGAND program [8] to provide values for the equilibrium dissociation constant (K_d). The program has been modified by us to calculate the Hill coefficient. Geometric means of the K_d values [9] are presented, and the standard error of the geometric mean was calculated as described [10]. Results represent mean values of at least three independent experiments, each performed in duplicate.

Results and Discussion

Competition between [^{3}H]QNB and the antidepressant compounds (Table 1) showed that at the m1 subtype the most potent drugs were amitriptyline > dothiepin > doxepin = nortriptyline; at the m2 receptor, amitriptyline > imipramine > nortriptyline = dothiepin; at the m3 receptor, amitriptyline > dothiepin > nortriptyline; at the m4 receptor, amitriptyline > dothiepin > doxepin = nortriptyline; and at the m5 receptor, amitriptyline > doxepin > imipramine. Our previous studies, which did not distinguish muscarinic receptor subtypes, found that amitriptyline was the most potent antidepressant at the muscarinic receptor in human brain tissue [11]. The least potent antidepressants at all five subtypes were adinazolam, venlafaxine, etoperidone, trazodone, and bupropion. For all practical purposes, these compounds are without any

* Abbreviations: CHO-K1, Chinese hamster ovary cells; and [^{3}H]QNB, [^{3}H]quinuclidinyl benzilate.

Table 1. Antidepressants and human muscarinic receptor subtypes

Drug	Human muscarinic receptor subtype				
	m1	m2	m3	m4	m5
			K_d* (nM)		
Adinazolam	>35,000	>35,000	>35,000	> 35,000	>35,000
Amitriptyline	14.7 ± 0.9	11.8 ± 0.8	12.8 ± 0.4	7.2 ± 0.5	15.7 ± 0.1
Bupropion	>35,000	>35,000	>35,000	>35,000	>35,000
Desipramine	110 ± 20	540 ± 10	210 ± 20	160 ± 10	143 ± 3
Desmethylsertraline	1,650 ± 90	3,300 ± 300	3,500 ± 200	3,100 ± 300	3,800 ± 100
Dothiepin	18 ± 3	109 ± 8	38 ± 2	61 ± 4	92 ± 3
Doxepin	38 ± 6	160 ± 10	52 ± 3	82 ± 8	75 ± 4
Etoperidone	>35,000	>35,000	>35,000	>35,000	>35,000
Femoxetine	92 ± 9	150 ± 5	220 ± 10	470 ± 20	400 ± 10
Fluoxetine	1,030 ± 90	2,700 ± 200	1,000 ± 100	2,900 ± 200	2,700 ± 100
Imipramine	42 ± 4	88 ± 7	60 ± 10	112 ± 5	83 ± 9
Lofepramine	67 ± 8	330 ± 60	130 ± 10	340 ± 20	460 ± 30
Norfluoxetine	1,200 ± 200	4,600 ± 800	760 ± 80	2,600 ± 100	2,200 ± 300
Nortriptyline	40 ± 3	110 ± 20	50 ± 3	84 ± 5	97 ± 2
Paroxetine	300 ± 40	340 ± 30	80 ± 10	320 ± 20	650 ± 20
QNB†	0.044 ± 0.002	0.030 ± 0.002	0.080 ± 0.003	0.037 ± 0.002	0.065 ± 0.005
Sertraline	1,300 ± 100	2,100 ± 80	1,300 ± 100	1,400 ± 100	1,900 ± 100
Trazodone	>35,000	>35,000	>35,000	>35,000	>35,000
Venlafaxine	>35,000	>35,000	>35,000	>35,000	>35,000

* Geometric means of data are presented. Unless noted otherwise, compounds for which SEMs are presented were tested in at least three independent experiments.

† This is not an antidepressant, but is the non-radioactive form of the radioligand used in the binding assays.

Table 2. Antihistamines and human muscarinic receptor subtypes

Drug	Human muscarinic receptor subtype				
	m1	m2	m3	m4	m5
			K_d* (nM)		
Cyproheptadine	12 ± 2	7 ± 1	12 ± 2	8 ± 1	11.8 ± 0.8
Mequitazine	5.6 ± 0.8	14 ± 1	5.3 ± 0.4	11.1 ± 0.6	11.0 ± 0.8
Orphenadrine	48 ± 2	213 ± 4	120 ± 10	170 ± 5	129 ± 7

* Geometric means ± SEM of data are presented. Compounds were tested in at least three independent experiments.

antimuscarinic activity. Hill coefficients for all compounds tested were close to one (data not shown), a result consistent with binding of these drugs to a single class of non-cooperative binding sites.

Competition between [^{3}H]QNB and the antihistaminics (Table 2) showed that mequitazine, which has structural features resembling both a tricyclic antidepressant and quinuclidinyl benzilate, had the highest affinity at the m1 and m3 subtypes. Cyproheptadine was the most potent antihistaminic at the m2 and m4 receptors. Mequitazine and cyproheptadine were equipotent at the m5 subtype. At all five muscarinic receptors orphenadrine was the least potent.

If receptor selectivity of a compound is having a K_d value at one receptor subtype that is less than one-tenth of that at another receptor, then no antidepressant or antihistaminic showed selectivity among the five subtypes. This result was not unexpected when considering the high degree of sequence homology among the receptors subtypes [3, 4]. In addition, it is not likely that these receptors expressed in the CHO-K1 cells have lost their selectivity. In our previous study of neuroleptics and antimuscarinics using the same cell lines, we found subtype selectivity for several drugs [5]. Thus, our data on the lack of muscarinic subtype selectivity of antidepressants support the conclusions of McKinney *et al.* [12] and not those of Nomura *et al.* [13].

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