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Antagonism by antidepressants of muscarinic acetylcholine receptors of human brain

Esam El-Fakahany[1] & Elliott Richelson

Departments of Psychiatry and Pharmacology, Mayo Foundation, Rochester, MN 55905, U.S.A.

1 Twenty-two compounds classified as antidepressants, metabolites of antidepressants or putative antidepressants were assayed for their ability to antagonize the binding of (−)-[^{3}H]-quinuclidinyl benzilate to muscarinic receptors in homogenates of human caudate nucleus.

2 Sixteen of these compounds were assayed for their ability to antagonize carbachol-stimulated cyclic guanosine 3′,5′-monophosphate (cyclic GMP) synthesis by intact murine neuroblastoma cells (clone N1E-115).

3 Equilibrium dissociation constants (K_Ds) for these drugs and the muscarinic receptors of human brain spanned over 4 orders of magnitude, with the tertiary amine tricyclic antidepressant, amitriptyline (K_D = 18 nM) being the most potent compound tested and trazodone (K_D = 324 μM) the least potent.

4 There was a significant correlation between the data for human and murine receptors and for eight compounds (imipramine, desipramine, maprotiline, mianserin, 3-chloro-2-hydroxyimipramine, amoxapine, 2-hydroxyimipramine and iprindole). K_D values measured by the two techniques were not significantly different.

Introduction

Data on the relative incidence of various side effects caused by antidepressants and other psychotherapeutic drugs is clinically useful information. Until relatively recently, only those side effects reported by patients provided a source of this information. Now data derived *in vitro* on the affinity of a drug-receptor interaction has proved useful for predicting the relative incidence of certain adverse effects caused by psychotropic drugs.

A number of different laboratories including our own have used radioligand binding assays to determine the affinities of antidepressants (see for example, Snyder & Yamamura, 1977; Golds, Przylso & Strange, 1980; Hall & Ogren, 1981) for several different neurotransmitter receptors in brains from mostly non-human species. We have in addition determined affinities for antidepressants and histamine H_1- and muscarinic receptors with biological assays, using intact murine neuroblastoma cells (Richelson & Divinetz-Romero, 1977; Richelson, 1978) and the guinea-pig isolated ileum (Figge, Leonard & Richelson, 1979). However, the question frequently arises whether data derived from studies with non-human tissues is applicable to human brain, the true site of action of these psychotherapeutic drugs. We have therefore undertaken a study to obtain equilibrium dissociation constants for antidepressants and several different neurotransmitter receptors of human brain using radioligand binding techniques. We now describe the results for antidepressants and muscarinic receptors and, in addition, present equilibrium dissociation constants for most of the compounds and muscarinic receptors of murine neuroblastoma cells using receptor-mediated cyclic guanosine 3′,5′-monophosphate (cyclic GMP) synthesis for our biological assay. The results show a favourable correlation between the data derived from these disparate species and approaches and, in addition, show a marked variation in the antimuscarinic potency of antidepressant compounds.

Methods

Radioligand binding assay

Human brains without disease were obtained at the time of autopsy. The time that elapsed between death

[1]Present address: Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201, U.S.A.

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and the removal of the brain ranged from 4 to 6 h. The brain was quickly dissected on ice by a neuropathologist into individual regions and stored frozen at −70°C until use. Tissue from the caudate nucleus was homogenized with a Polytron tissue homogenizer (Brinkman Instruments; setting no. 8, 30 s) and 20 vol of ice-cold Na^+-K^+ phosphate (50 mM, pH 7.4) and the resulting homogenate was centrifuged at 30,000 *g* for 15 min. The pellet was resuspended in the same volume of fresh buffer, homogenized and centrifuged as before and the final pellet was resuspended in buffer to give a 1% (w/v) homogenate. For the binding assay, 100 µl of tissue homogenate was incubated with 0.1 nM (−)-[^{3}H]-quinuclidinyl benzilate (QNB) with increasing concentrations of unlabelled (±)-QNB or antidepressant (usually 12 different concentrations of each compound) in a final volume of 1 ml. Only the (−)-form of QNB was assumed to be active (Strange, Birdsall & Burgen, 1978). Specific binding was derived empirically by the curve-fitting programme described below and was always greater than 90% of the total binding. Incubation was at 37°C for 60 min after which time the assay was terminated by filtering the suspension through Whatman GF/B filters (Whatman, England) under vacuum, followed by rapidly washing the filters four times with ice-cold Na^+-K^+ phosphate buffer (50 mM, pH 7.4). After 5 min the filters were placed in scintillation vials to which was added 7 ml of scintillation mixture. Radioactivity was determined at least 8 h later in a Searle Isocap/300 liquid scintillation counter at an average efficiency for tritium of 37%.

Biological assay with cultured cells

Murine neuroblastoma cells (clone N1E-115; subculture 10–20) were cultured and assayed for receptor-mediated cyclic [^{3}H]-GMP formation by prelabelling cells with [^{3}H]-guanine as described previously (Richelson, Prendergast & Divinetz-Romero, 1978). In order to assure equilibrium conditions between the receptor and antagonist, drugs and related compounds were incubated with cells for 30 min before the addition of agonist for 30 s. Dose-ratios were determined for at least three different concentrations of antagonist.

Analysis of data

Radioligand binding data were analysed to obtain equilibrium dissociation constants (K_Ds) with the use of the programme LIGAND (Munson & Rodbard, 1980) on a Cyber computer (Control Data Corporation, Minneapolis, MN) using our Hewlett-Packard 9845B desktop computer (Cupertino, CA) interfaced by phone modem to the Cyber. For all compounds except nomifensine and trazodone, K_Ds were obtained in at least three independent experiments and these constants were averaged for presentation (Table 1).

Dose-response curve data from the biological assay were fitted to an empirical function by employing the least squares methods of Waud & Parker (1971) using a programme BIGSIG on the HP9845B. Dose-ratio data were further analysed by direct plot (Tallarida, Cowan & Adler, 1979) to obtain equilibrium dissociation constants ± s.e. unless otherwise noted.

Materials

(−)-[^{3}H]-quinuclidinyl benzilate (26.8 Ci/mmol) was obtained from New England Nuclear Corporation (Boston, MA) and [^{3}H]-guanine from Amersham/Searle Corporation (Arlington Heights, IL). Carbamylcholine chloride was purchased from Sigma Chemical Co. (St Louis, MO). We thank Dr A. Manian, N.I.M.H., for supplying didesmethylimipramine hydrochloride, 3-chloro-2-hydroxyimipramine hydrogen oxalate and 2-hydroxyimipramine hydrochloride; and the following companies for supplying antidepressants: Ayerst Labs (butriptyline hydrochloride); Burroughs-Wellcome Co. (buproprion); CIBA-Geigy Corp. (clomipramine hydrochloride, imipramine hydrochloride and maprotiline hydrochloride); Eli Lilly and

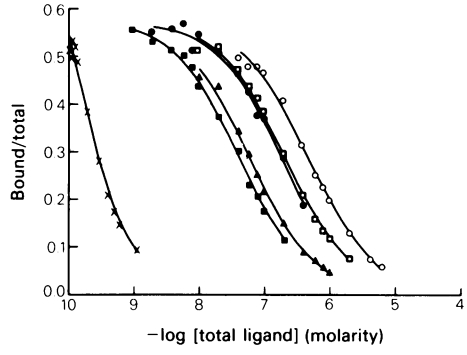

Figure 1 Competition by antidepressants for [^{3}H]-quinuclidinyl benzilate binding to muscarinic receptors of human caudate nucleus. This graph which presents the results of one experiment was generated by computer with the use of the programme called LIGAND (Munson & Rodbard, 1980). The concentration of (−)-[^{3}H]-quinuclidinyl benzilate was 0.1 nM and the concentrations of unlabelled compounds were varied as indicated. Although (±)-quinuclidinyl benzilate was used to displace (−)-[^{3}H]-quinuclidinyl benzilate, we assumed that only the (−)-form was active. (x) Quinuclidinyl benzilate; (□) imipramine; (○) desipramine; (▲) protriptyline; (●) doxepin; (■) amitriptyline.

Table 1 Antidepressants and related compounds: equilibrium dissociation constants (K_D) for muscarinic acetylcholine receptors of human brain and murine neuroblastoma cells

Compound	*Human brain* $K_D \pm$ s.e.mean (nM)	Hill coefficient ($n_H \pm$ s.e.mean)	*Murine neuroblastoma* $K_D \pm$ s.e. (nM)
Amitriptyline[a]	18 ± 1	1.00 ± 0.04	25 ± 2
Protriptyline[b]	25 ± 1	1.00 ± 0.04	62 ± 5
Butriptyline[a]	35 ± 6	1.07 ± 0.09	63 ± 6
Clomipramine[a]	37 ± 4	1.09 ± 0.16	220 ± 10[c]
Trimipramine[a]	58 ± 12	1.05 ± 0.08	120 ± 40[c]
Doxepin[a]	80 ± 6	1.01 ± 0.07	41 ± 7
Imipramine[a]	90 ± 3	0.98 ± 0.04	85 ± 10[c]
Nortriptyline[b]	150 ± 30	0.96 ± 0.06	270 ± 20
Desipramine[b]	198 ± 14	0.89 ± 0.01	270 ± 20[c,d]
Maprotiline	570 ± 120	1.02 ± 0.10	410 ± 40[d]
Didesmethylimipramine	590 ± 210	0.93 ± 0.06	2400 ± 400[c]
Mianserin	820 ± 110	1.04 ± 0.04	800 ± 140[d]
3-Chloro-2-hydroxyimipramine	870 ± 30	1.09 ± 0.02	780 ± 170[c,d]
Amoxapine	1000 ± 150	1.01 ± 0.05	970 ± 110[d]
2-Hydroxyimipramine	1210 ± 190	0.88 ± 0.02	990 ± 160[c,d]
Fluoxetine	2000 ± 400	0.92 ± 0.03	–
Iprindole	2100 ± 350	0.96 ± 0.06	3030 ± 900[d]
Nisoxetine	3200 ± 50	0.96 ± 0.06	–
Bupropion	48000 ± 9000	1.04 ± 0.07	–
Viloxazine	54000 ± 7000	1.10 ± 0.08	–
Nomifensine	250000	–	–
Trazodone	324000	–	–

[a] Tricyclic antidepressants with tertiary amine sidechains; [b] tricyclic antidepressants with secondary amine sidechains.
[c] Data from Petersen & Richelson (1982) and presented as $K_D \pm$ s.e.mean.
[d] Not significantly different from value obtained with human brain tissue in two-tailed t test where the criterion for significance was $P < 0.05$.

Co. (nortriptyline hydrochloride, fluoxetine, and nisoxetine hydrochloride); Hoechst-Roussel (nomifensine maleate); Ives Labs (trimipramine maleate); Lederle Labs (amoxapine); Mead Johnson and Company (trazodone hydrochloride); Merck Sharp and Dohme (amitriptyline hydrochloride and protriptyline hydrochloride); Organon Inc. (mianserin); Pfizer Inc. (doxepin); Stuart Pharmaceuticals (viloxazine); USV Pharmaceuticals (desipramine hydrochloride); and Wyeth Labs (iprindole hydrochloride). Hoffman-La Roche kindly supplied (±)-quinuclidinyl benzilate.

Results

Radioligand binding to human brain receptors

The mean equilibrium dissociation constant (K_D) for (−)-[^{3}H]-quinuclidinyl benzilate was (± s.e.mean, $n = 10$) $5.8 \pm 1.4 \times 10^{-11}$ M with a Hill coefficient ($n_H \pm$ s.e.mean) of 1.10 ± 0.11. Thus, the binding of this radioligand to muscarinic receptors of human brain (caudate nucleus) (Figure 1) obeyed the law of mass action and the K_D for this interaction was similar to the values obtained by others (Wastek & Yamamura, 1978).

The antidepressants and related compounds (i.e., putative antidepressants and some metabolites of antidepressants) were competitive antagonists of (−)-[^{3}H]-quinuclidinyl benzilate (e.g., Figure 1) and the derived K_D values spanned over 4 orders of magnitude, with the tertiary amine tricyclic antidepressant amitriptyline ($K_D = 18$ nM) being the most potent compound tested and trazodone ($K_D = 324$ μM) the least potent (Table 1). For comparison, the K_D for the classical antimuscarinic, atropine, was 1.8 nM.

Receptor-mediated cyclic GMP synthesis by murine neuroblastoma cells

Equilibrium dissociation constants for the antidepressants and related compounds were determined directly from the dose-ratio analysis of dose-response curves for carbachol (e.g., Figure 2) or in most cases by direct plot of these dose-ratio data vs. concentration of antagonist (e.g., Figure 3). For the

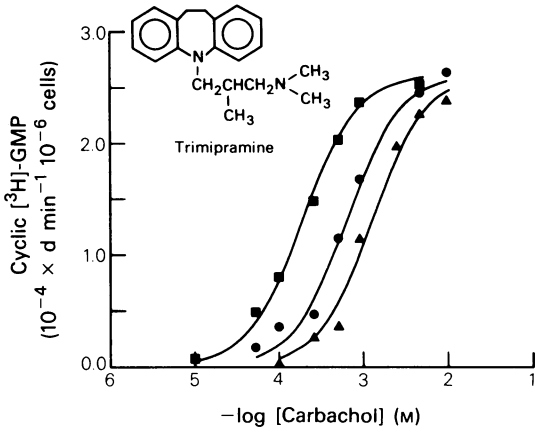

Figure 2 Effect of trimipramine on muscarinic receptor-mediated cyclic [^{3}H]-GMP synthesis by murine neuroblastoma cells (clone N1E-115). For this experiment there were approx. 2×10^5 cells per assay. Basal values which averaged 4600 d min^{-1} 10^{-6} cells were subtracted from data which were fit by computer to an empirical function as described by Waud & Parker (1971). Dose-ratio analyses gave an average $K_D = 9.5 \times 10^{-8}$ M. (■) Control; (●) 0.25 μM trimipramine; (▲) 0.5 μM trimipramine.

sixteen compounds for which K_D values were determined by both the radioligand binding assay and the cyclic GMP assay, there was a significant correlation ($P = 0.001$) for the regression of $-\log K_D$ (log affinity) for murine neuroblastoma receptors on $-\log K_D$ for human caudate nucleus receptors

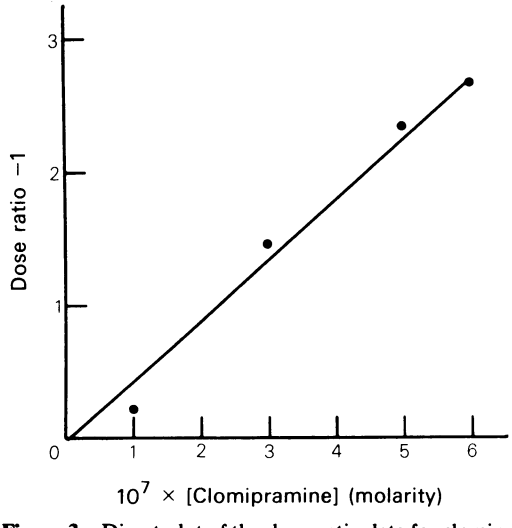

Figure 3 Direct plot of the dose-ratio data for clomipramine. The analysis by direct plot of dose-ratio data is described by Tallarida *et al.* (1979). The result of this analysis was $K_D \pm$ s.e. $= 2.20 \pm 0.08 \times 10^{-7}$ M.

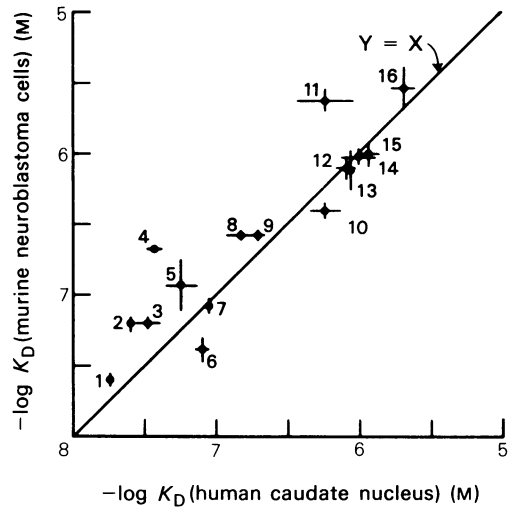

Figure 4 Equilibrium dissociation constants for antidepressants and related compounds and human brain muscarinic receptors correlated with those for murine muscarinic receptors of murine neuroblastoma cells: (1) Imipramine; (2) desipramine; (3) protriptyline; (4) doxepin; (5) amitriptyline; (6) amoxapine; (7) maprotiline; (8) trimipramine; (9) nortriptyline; (10) clomipramine; (11) mianserin; (12) butriptyline; (13) iprindole; (14) 2-OH-imipramine; (15) 3-Cl-2-OH-imipramine; (16) didesmethylimipramine.

(Y = 1.15 + 0.81X). In addition, for eight of these compounds (Table 1 and Figure 4), the K_D derived from one assay was not significantly different from the K_D derived from the second assay.

Discussion

These results largely confirm and extend previously reported results on the antimuscarinic property of antidepressants and related compounds (Brimblecombe & Green, 1967; Atkinson & Ladinsky, 1972; Snyder & Yamamura, 1977; Fjalland, Christensen & Hytell, 1977; Rehavi, Maayani & Sokolovsky, 1977; Shein & Smith, 1978; Golds *et al.*, 1980; Hyslop & Taylor, 1980; Hall & Ogren, 1981). However, no previously published study has made use of muscarinic receptors of human brain to determine the equilibrium dissociation constants for antidepressants and these receptors.

In general, the tricyclic antidepressants with tertiary amine side chains (Table 1) were the most potent compounds tested with one exception: protriptyline, a tricyclic compound with a secondary amine side chain was only slightly less potent than amitriptyline (Table 1 and Figure 5). The four least potent compounds (Table 1 and Figure 5) were structurally

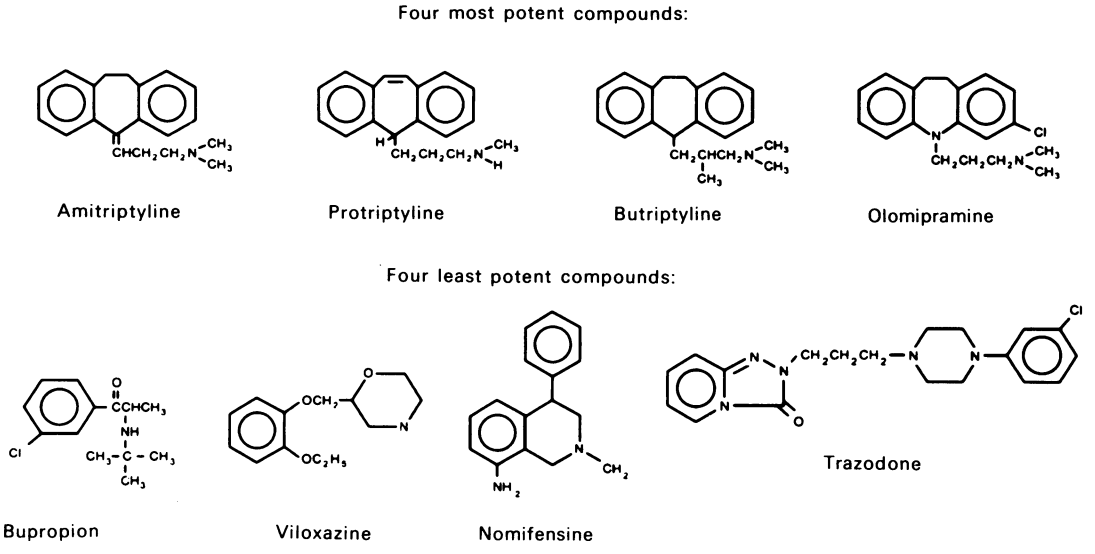

Figure 5 Structures of the four most potent and four least potent antidepressants at muscarinic receptors of human brain.

very different from the tricyclic drugs. Finally, as noted previously (Petersen & Richelson, 1982) ring hydroxylation of the tricyclic antidepressants, clomipramine (3-chlorimipramine) and imipramine, compounds with tertiary amine side chains, markedly reduced their antimuscarinic potencies for the human brain receptors by greater than 20 and 10 fold, respectively (Table 1).

Of the twenty-two compounds tested for their effects on muscarinic receptors of human brain (Table 1), three, fluoxetine, iprindole and nisoxetine, can be classified as probably effective antidepressants (Feighner, 1981); and three, didesmethylimipramine, 3-chloro-2-hydroxyimipramine and 2-hydroxyimipramine—can be classified as metabolites of antidepressants with possible antidepressant efficacy (Potter, Calil, Manian, Zavadil & Goodwin, 1979). The remainder have been established as antidepressants (Morris & Beck, 1974; Feighner, 1981).

It was interesting to compare our results from radioligand binding studies of human brain with those from the biological studies with murine neuroblastoma cells (Table 1 and Figure 4). Many different laboratories have studied muscarinic receptor-mediated cyclic GMP synthesis by cells of murine neuroblastoma clone N1E-115 and Strange, Birdsall & Burgen (1977) have shown for a small series of compounds that identical results are obtained with this assay as compared to those obtained with radioligand binding assays of muscarinic receptors of these cells or even rat brain homogenates. We found for 16 compounds a significant correlation of the murine neuroblastoma data with the human brain data ($P = 0.001$) and for eight compounds (imipramine, desipramine, maprotiline, mianserin, 3-chloro-2-hydroxyimipramine, amoxapine, 2-hydroxyimipramine and iprindole) K_D values measured by the two methods were not significantly different.

Shein & Smith (1978) have determined the affinities of antidepressants for muscarinic receptors from their ability to antagonize carbachol-stimulated contractions of the guinea-pig ileum. Eleven of the compounds that they studied are in common with our list and a regression of the log affinity for the guinea-pig data on the log affinity for the human brain data gave the equation $Y = 0.54 + 1.05X$ ($P = 0.0001$). In addition, Golds *et al.* (1980) using radioligand binding techniques and rat brain receptors, obtained equilibrium dissociation constants for a number of antidepressants. For the seven compounds in common between their study and ours (excluding nomifensine and viloxazine, precise data for which are lacking), the regression gave the equation $Y = 0.47 + 0.90X$ ($P = 0.0013$). We conclude from these analyses that there are no apparent species differences between human, mouse, rat, and guinea-pig in the affinities of antidepressants for muscarinic receptors.

There is some evidence to support the hypothesis that the antimuscarinic property of antidepressants is responsible for their mood-elevating effect (Janowsky, El-Yousef, Davis & Scherke, 1972; Davis, Berger, Hollister & Barchas, 1978). Abuse of antimuscarinic drugs for their euphoriant and other

effects also supports this idea (Bluhm & Koller, 1981). However, rigorously controlled studies on the efficacy of antimuscarinic agents in treating depression are lacking and need to be done to know whether muscarinic blockade is important for antidepressant efficacy.

Evidence presented here does not support this hypothesis since there is such a broad range of antimuscarinic potencies among the antidepressants with some compounds being practically devoid of activity (Table 1). However, it is more likely that the antimuscarinic effect of these drugs *in vitro* reflects their propensity to cause adverse effects in patients such as dry mouth, blurred vision, urinary retention and constipation. With the knowledge of the potencies of these drugs at muscarinic receptors the clinician, by choosing the appropriate drug, can avoid or minimize these side effects when giving these drugs to patients.

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