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Effect of Antidepressant Agents on β-Adrenergic Receptor and Neurotransmitter Regulatory Systems

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MEYERSON, L. R., H. H. ONG, L. L. MARTIN AND D. B. ELLIS. *Effect of antidepressant agents on β-adrenergic receptor and neurotransmitter regulatory systems.* PHARMAC. BIOCHEM. BEHAV. 12(6) 943–948, 1980.—The effects of established and several novel antidepressant agents on brain monoamine oxidase A and B; high affinity synaptosomal uptake of norepinephrine, dopamine and serotonin; and β-adrenergic receptor kinetics evaluated by (^{3}H)-dihydroalprenolol binding to cortical membranes are described. Extremely weak *in vitro* inhibitory effects on rat brain mitochondrial MAO-type A or B were observed with P74-1197 (+) or (−) and HP-505, both 3-aryl-spiroisobenzofuranpiperidines, P77-2984 a 3-aryl-spirobenzothiophenepiperidine derivative, LM-5008 an indolylethylpiperidine, desipramine, nisoxetine and P76-2543 a 4-aryl-1,3 benzodiazapine. As anticipated, deprenyl showed potent substrate selective inhibition of MAO type B. LM-5008, P74-1197(+) and P77-2984 were potent selective inhibitors of serotonin synaptosomal uptake while nisoxetine, P76-2543 and P74-1197(−) appeared to preferentially inhibit reuptake of norepinephrine. The kinetics (B_{max} and K_D) of (^{3}H)-dihydroalprenolol binding were also studied following chronic administration of these same drugs (10 mg/kg, b.i.d.). After 10 days of treatment, heterogeneous results were obtained in that some compounds elicited changes in receptor density and dissociation constant while others, such as nisoxetine, produced no kinetic alterations. While present biochemical antidepressant tests utilized in this study are designed to evaluate modulations of aminergic systems in terms of neurotransmitter availability, fluxes in concentration and attendant receptor recognition site sensitivities, the underlying mode(s) of action at the cellular level still require further clarification.

β-Adrenergic receptor Antidepressants Monoamine uptake inhibition Monoamine oxidase Dihydroalprenolol binding Receptor sensitivity

PERTINENT features of antidepressant agents are their inhibitory actions on the neuronal reuptake mechanisms of dopamine (DA), norepinephrine (NE) and serotonin (5-HT), and/or inhibition of monoamine oxidase (MAO). Tricyclic drugs, for example, are proposed to augment concentrations of certain neuroamines at synaptic junctions by preventing reuptake into their respective nerve endings. Drugs demonstrating MAO inhibition, on the other hand, curtail the catabolism of monoaminergic neurotransmitters resulting in a presynaptic accumulation of these transmitter substances and subsequently a greater concentration of "releasable" amine. However, these biochemical-pharmacologic effects occur shortly after drug administration while clinical reversal of depression usually requires several weeks of drug therapy [26].

Proposals to explain this apparent time-course discrepancy have focused upon gradual developing adaptations at the level of the post-synaptic neurotransmitter receptors [2, 4, 10, 14, 15, 29, 34, 35, 42, 44]. Several investigators have shown that antidepressants administered chronically can reduce the catalytic ability of norepinephrine to stimulate cAMP production in rat brain slices [14, 15, 34, 35, 42, 44]. Additional workers have demonstrated that chronic antidepressant administration is accompanied by the development of subsensitivity (decreased density) of β-adrenergic recognition sites labeled by (^{3}H)-dihydroalprenolol (DHA) [2, 4, 10, 29, 44]. Most recently, serotonergic receptor systems have been implicated in the neurochemical actions of antidepressant agents [31,37]. These studies revealed that the maximal number of serotonin binding sites were significantly decreased following chronic administration of selected antidepressants.

Thus, it is pertinent to define the full biochemical spectrum of various classes of compounds possessing antidepressant activity. The present investigation describes the effects of several new and established antidepressant agents on MAO types A and B; high affinity synaptosomal uptake of NE, DA and 5-HT; and β-adrenergic receptor kinetics evaluated by (^{3}H)-DHA binding to cortical membranes.

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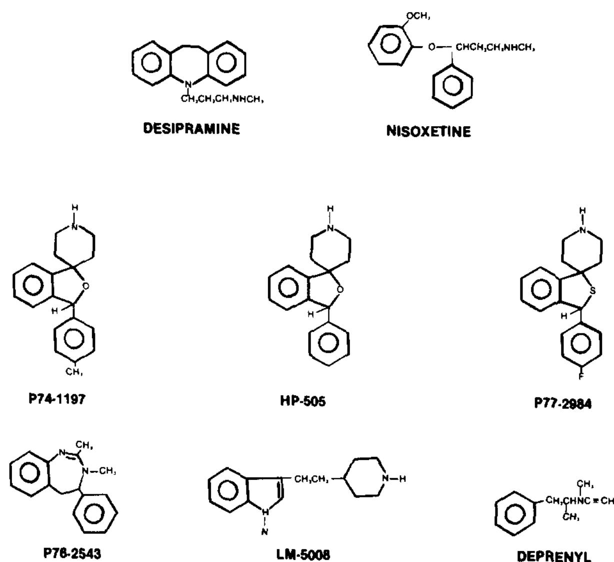

FIG. 1. Structural representation of antidepressant compounds investigated in this study.

METHOD

Drugs and Reagents

Desmethylimipramine (desipramine HCl, DMI) was obtained from USV Pharmaceutical Corp., Tuckahoe, NY. Deprenyl (E-250) was supplied by Dr. J. Knoll, Semmelweiss University, Budapest, Hungary. LM-5008, 4-[2-(3-indolyl)ethyl]-piperdine, was obtained from Pharmuka, Paris, France. Nisoxetine, (±)-3-(o-methoxyphenoxy)-N-methyl-3-phenylpropylamine was kindly furnished by Eli Lilly and Company, Indianapolis, IN. HP-505, 3-phenylspiro[isobenzofuran-1(3H)-4′-piperidine]; P77-2984, 3-(4-fluorophenyl)-1, 3-dihydrospiro (benzo[c]thiophene-1,4′-piperidine); P77-2543, 4,5-dihydro-2,3-dimethyl-4-phenyl-3H-1,3-benzodiazepine; and the optical isomers P74-1197 (+) and (−), 3-(4-methylphenyl)spiro[isobenzofuran-1(3H),4′-piperidine] were synthesized at Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ. The radiochemicals, 5-hydroxytryptamine binoxalate [2-^{14}C]; 5-hydroxytryptamine creatinine sulfate, 5-[1,2-^{3}H(N)]; dopamine [^{3}H(G)]; norepinephrine, Levo-[7,8-^{3}H(N)]; phenethylamine hydrochloride, β-[ethyl-1-^{14}C] and dihydroalprenolol hydrochloride, Levo-[propyl-1,2,3,^{3}H] were purchased from New England Nuclear Corp., Boston, MA. 5-Hydroxytryptamine creatinine sulfate, phenethylamine hydrochloride, dopamine hydrochloride and dl-propranolol were from Sigma Chemical Co., St. Louis, MO. All other reagents used were of the highest quality commercially available.

The chemical structures of the antidepressant agents used in this study are shown in Fig. 1.

Monoamine Oxidase Assay

Male Wistar rats weighing between 150–250 g were decapitated and brains were rapidly removed, rinsed and chilled in 0.1 M sodium phosphate buffer, pH 7.4. A 10% (w/v) homogenate was prepared in the same buffer with twelve strokes of a Potter-Elvehjem homogenizer (Kontes, Vineland, NJ), and used for enzymatic assays. MAO type A or B was monitored by a radioisotopic assay as previously described [9,25] using (^{14}C) serotonin or (^{14}C)phenethylamine as type A or B substrates, respectively. The incubation mixtures contained the following components: 50 mM sodium phosphate buffer (pH 7.4); enzyme preparation (2–4 mg protein); 0.5 mM serotonin or 0.12 mM phenethylamine (specific radioactivity 0.1 μCi/μmole); various concentrations of antidepressant drugs; and deionized-distilled water to a final volume of 1.0 ml. Reaction mixtures were preincubated for 10 min before the addition of substrate. Following a 30-min incubation at 37°C, 0.3 ml of 2 N HCl was added to terminate the reaction. Substrates were then added to reaction blanks after the termination of the reaction. The deaminated metabolites were extracted into 7 ml of diethylether and 4-ml aliquots of the organic layer were removed and counted in 10 ml of scintillation fluid (3a70B, counting cocktail, Research Products, Inc., Elk Grove Village, IL). Radioactivity was determined with a Beckman LS-355 liquid scintillation system. IC_{50} values with mean ± SEM were obtained from Litchfield Wilcoxin log-probit analysis.

Amine Uptake Assays

The synaptosomal reuptake of tritiated NE, DA or 5-HT was measured by modifications of previously described methods [39]. Male Wistar rats (150–200 g) were sacrificed by decapitation and whole brains rapidly removed and either used entirely (NE and 5-HT studies) or regionally (corpus striatum for DA studies). Brain dissection was performed on a moistened filter paper disc placed on an inverted glass petri dish embedded in ice. Tissues from 5–6 rats were pooled, weighed and homogenized in 9 volumes of cold 0.32 M sucrose using a wide clearance Potter-Elvehjem homogenizer (Kontes, Vineland, NJ).

The homogenate was centrifuged at 1000 ×g for 10 min at 4°C to remove nuclei and cell debris as a pellet (P_1). The crude synaptosomal supernatant (S_1) was used for uptake studies within one hour. Aliquots of tissue preparation (20 mg tissue per original wet weight for 5-HT and NE assays and 10 mg tissue for DA uptake) were added to tubes containing cold oxygenated Krebs-Henseleit bicarbonate buffer containing iproniazid (0.1 mM) and dextrose (10 mM). Tritiated NE, DA or 5-HT (0.05 μM) were added followed by varied concentrations of drugs (10^{-4}–10^{-8}M). Tubes were mixed gently and incubated for 5 min at 37°C. Blanks consisted of samples kept at 0°C throughout the procedure.

Following incubation, all tubes were immediately centrifuged at 3000 ×g for 10 min to collect the tissue as a pellet. The resultant supernatant was aspirated off and 1 ml of solubilizing agent (Triton X-100 and 50% ethanol, 1:4 v/v) was added to each tube and mixed by vortex. An aliquot of each sample was withdrawn and transferred to counting vials containing 10 ml of scintillation fluid (3a70B counting cocktail, Research Products, Inc., Elk Grove Village, IL). Radioactivity was determined with a Beckman LS-355 liquid scintillation system. Active uptake was defined as the difference in radioactivity accumulated at 37°C and 0°C. Drug IC_{50} values are reported as the concentration of drug that inhibited the accumulation of (^{3}H)-amine by 50%. Values are expressed as mean ± SEM obtained from computer derived Litchfield-Wilcoxin log-probit analyses.

Chronic Treatment of Animals

Male Wistar rats (150–250 g) were housed in a light (12-hr cycle) and temperature (23°C) controlled room with free access to food and water. Drugs depending upon solubility

TABLE 1

EFFECT OF VARIOUS ANTIDEPRESSANT AGENTS ON RAT BRAIN MAO ACTIVITY WITH SEROTONIN (TYPE A) AND PHENETHYLAMINE (TYPE B) AS SUBSTRATES

Compound	IC_{50} MAO-A (mM)	IC_{50} MAO-B (mM)
P77-1197(+)	>1.0	>1.0
P77-1197(−)	>1.0	>1.0
HP-505	>1.0	>1.0
Desipramine	0.5 ± 0.04	0.7 ± 0.06
Nisoxetine	>1.0	>1.0
P76-2543	>1.0	>1.0
P77-2984	>0.5	>1.0
Deprenyl	0.02 ± 0.005	0.00042 ± 0.00013
LM-5008	0.5 ± 0.1	1.6 ± 0.4

Values given are mean ± SEM for three separate experiments.

were dissolved either in 0.9% NaCl or in 0.9% NaCl containing 20% dimethyl sulfoxide and intraperitoneally injected at a dosage of 10 mg/kg twice daily for a period of 10 days. Control animals received the corresponding vehicle. Twenty-four hours after the last injection, rats were sacrificed by decapitation and the cortices were rapidly removed, weighed, and placed in cold 0.9% NaCl. Tissues were then homogenized in 20 volumes of 0.05 M Tris buffer, pH 8.0, using a Tissumizer homogenizer (Tekmar Co., Cincinnati, OH) and centrifuged at 49,000 ×g for 15 min at 4°C in a Sorvall RC5 refrigerated centrifuge. The pellet was dispersed in 50 volumes of original buffer and immediately used in binding assays.

(^{3}H)-DHA Binding Assays

Beta-adrenergic binding was assayed by modifications of previously established procedures [1,8]. Reaction mixtures for (^{3}H)-DHA binding contained the following components in final concentrations: 10 mg tissue; 0.05 M Tris buffer, pH 8.0; (^{3}H)-DHA (0.25–4.0 nM) and deionized-distilled water to a final volume of 1 ml. Corrections were made for non-specific binding by assaying parallel incubations which contained 1 μM dl-propranolol. All samples were run in quadruplicate. After a 15-min incubation at 23°C, the reaction was terminated by filtration through prewetted Whatman 2.4 cm GF/B glass fiber filters. The filters were washed four times with 5 ml of cold 0.05 M Tris buffer to remove the unbound radioactive ligand. The filters were placed in scintillation vials containing 10 ml of scintillation fluid (3a70B counting cocktail, Research Products, Inc., Elk Grove Village, IL). The vials were refrigerated overnight and counted in a Beckman LS-355 liquid scintillation system. Data were analyzed by Scatchard plots [28] and the best fitting lines determined by least squares linear regression analysis.

Protein Determination

Protein concentrations were determined by the protein-dye binding method [7] utilizing the Bio-Rad Protein Assay Kit (BioRad Labs; Richmond, CA). Bovine gamma globulin was used as the protein standard.

RESULTS

Effects of Antidepressants on Brain MAO Type A and B

Extremely weak or negligible *in vitro* inhibitory effects on rat brain MAO type A or B were observed (Table 1) with P74-1197(+), P74-1197(−), HP-505, P77-2984, LM-5008, P76-2543, nisoxetine or desipramine. Deprenyl, inhibited the oxidation of the MAO type B substrate, phenethylamine ($IC_{50}=4.2\times10^{-7}$M) with greater potency compared to the MAO type A substrate, serotonin ($IC_{50}=2.0\times10^{-5}$M). This observation is consistent with the actions of substrate-preferred MAO type B inhibitors [16, 22, 25].

TABLE 2

EFFECT OF VARIOUS ANTIDEPRESSANT AGENTS ON THE IN VITRO SYNAPTOSOMAL REUPTAKE OF NOREPINEPHRINE DOPAMINE AND SEROTONIN*

Compound	IC_{50} NE (μM)	IC_{50} DA (μM)	IC_{50} 5-HT (μM)	IC_{50} NE / IC_{50} 5-HT
P74-1197(+)	0.30 ± 0.15	0.77 ± 0.35	0.022 ± 0.01	13.6
P74-1197(−)	0.20 ± 0.06	1.40 ± 0.70	1.90 ± 0.58	0.1
HP-505	0.34 ± 0.10	0.66 ± 0.15	0.19 ± 0.04	1.8
Desipramine	5.8 ± 2.5	7.80 ± 0.70	11.0 ± 2.0	0.5
Nisoxetine	0.29 ± 0.1	1.7 ± 0.5	3.3 ± 1.1	0.08
P76-2543	1.68 ± 0.64	5.25 ± 2.7	8.4 ± 1.8	0.2
P77-2984	0.15 ± 0.03	0.35 ± 0.04	0.027 ± 0.01	5.6
Deprenyl	>20.0	>20.0	>20.0	—
LM-5008	5.7 ± 1.2	9.6 ± 3.2	0.019 ± 0.02	300

*NE and 5-HT uptake studies were performed using whole brain synaptosomes while DA uptake was examined in striatal preparations. Values given are the mean ± SEM for three separate experiments.

Effects of Antidepressants on Synaptosomal Reuptake of Biogenic Amines

The concentrations of antidepressant agents inhibiting the uptake of (^{3}H)-NE,(^{3}H)-DA or (^{3}H)-5-HT into rat brain synaptosomes by 50% (IC_{50}) are shown in Table 2. P77-2984 was the most potent inhibitor of (^{3}H)-NE uptake into whole brain synaptosomes closely followed by P74-1197(−), nisoxetine, P74-1197(+), and HP-505. P76-2543, desipramine or LM-5008 did not exhibit the same potency of inhibition of (^{3}H)-NE uptake. Deprenyl in concentrations up to 20 μM had no effect on the uptake of (^{3}H)-NE. Modest inhibition of (^{3}H)-DA reuptake was observed with P77-2984, HP-505 and P74-1197(+) with IC_{50} values of 0.35, 0.66 and 0.77 μM, respectively. In inhibiting the reuptake of (^{3}H)-5-HT, extremely potent effects were observed with LM-5008, P77-2984 and P74-1197(+) with respective IC_{50} values of 0.019, 0.027 and 0.022 μM. Again deprenyl had no inhibitory effect on either (^{3}H)-DA or (^{3}H)-5-HT reuptake at concentrations up to 20 μM. By calculating the ratio of IC_{50}(NE)/IC_{50}(5-HT) an index of selectivity in terms of inhibitory potency can be derived. Thus, the larger the quotient the greater the selectivity in inhibiting the reuptake of 5-HT, while lower values indicate the preferential inhibition of NE uptake. In this light, LM-5008, P74-1197(+) and P77-2984 may be considered 5-HT reuptake inhibitors while nisoxetine and P74-1197(−) can be classified as, selective NE reuptake inhibitors. P77-2543, desipramine and HP-505 apparently possess little selectivity.

Effect of Antidepressants on Beta-Adrenergic Receptor Binding Kinetics

Following a 10-day chronic drug regimen, a significant

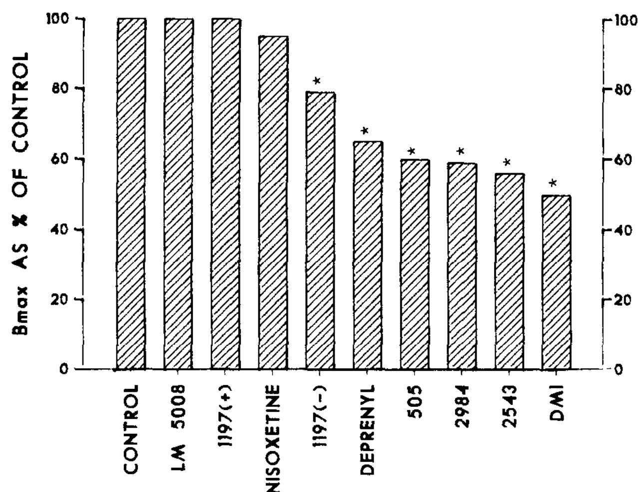

FIG. 2. Histogram representation of the effect of chronic antidepressant treatment (10 mg/kg, IP, B.I.D., for 10 days) on β-adrenergic receptor density (B_{max}). Data were derived from Scatchard analyses [32] and histogram results are the means of the Scatchard X-intercepts reflecting B_{max} normalized to control=100. Vehicle injected control B_{max} values for a typical experiment were 6.5±0.5 pmoles/g tissue and control K_D values were 0.8±0.2 nM. For details of tissue preparation and assay conditions see METHOD. *$p<0.05$ level of significance.

decrease was observed in the specific binding of (^{3}H)-DHA in the cortices of animals treated with DMI, HP-505, deprenyl, P76-2543, P77-2984, or P74-1197(−). To determine whether the reduced binding of (^{3}H)-DHA in animals chronically treated with these antidepressant agents was due to a decreased population of binding sites (B_{max}) or to a change in affinity (K_D) of the binding sites for the ligand, tissue samples were incubated with varying concentrations of (^{3}H)-DHA and analyzed by the method of Scatchard [32].

Figure 2 is a histogram of Scatchard X-intercepts of relative B_{max} values compared to controls. Maximum number of cortical binding sites was significantly decreased in rats treated with DMI(50% reduction), P76-2543(44%), P77-2984(41%), HP-505(40%), deprenyl(35%), and P74-1197(−)(21%). Decreases in K_D values for (^{3}H)-DHA were observed in cortical tissue of rats treated with P76-2543 and P77-2984 while all other drugs administered chronically had no significant effect on ligand dissociation constants. Since micromolar concentrations of antidepressant drugs used in this study had no appreciable effect in inhibiting the specific binding of (^{3}H)-DHA (data not shown) it is unlikely that residual drug effects or direct receptor-drug interactions occur.

DISCUSSION

Various biochemical approaches may be utilized to evaluate the propensity of a compound to elicit antidepressant effects. A multitude of observations of adrenergic involvement, including both suppression and facilitation have been implicated regarding the chronicity of antidepressant administration. Tyrosine hydroxylase activity, a presynaptic indicator, has been shown to be reduced after long-term antidepressant treatment [36]. As a consequence to this, brain NE concentrations may be reduced [28]. Furthermore, the neuronal firing rate has been shown to be decreased after administration of tricyclic antidepressants [40]. Opposite to these reported effects are the observations that NE-turnover is increased [33], amine reuptake [18,21] and MAO [22] are inhibited, and reduced presynaptic α-receptor sensitivity occurs [11]. Thus, with the observed diversity of action of antidepressants, the net *in vivo* effect following a chronic antidepressant regimen remains unknown. Proposals based upon hippocampal cell activity suggest that the net effect of long term DMI treatment is antagonistic to noradrenergic function [19].

Compensatory responses to increased neurotransmitter molecules at noradrenergic nerve terminals most probably result in sensitivity changes in postsynaptic β-receptor kinetics [2, 4, 10, 29, 44]. Usually, investigators have observed only B_{max} changes in cortical (^{3}H)-DHA binding following antidepressant administration. The present data reflect in some cases alterations in both B_{max} and K_D values, either of which would represent drug-mediated changes in postjunctional β-adrenergic recognition sites.

Along with catecholaminergic systems, serotonergic systems have been implicated in the pathogenesis of the depressive syndrome. Data have accumulated relating decreased central serotonergic function to depressive illness in terms of 5-HT and 5-HIAA deficiencies [6, 11, 12, 23, 38]. Indeed, recent reports indicate a significant reduction in the density of serotonin binding sites following a long-term antidepressant paradigm [31,37].

While present biochemical antidepressant tests are designed to evaluate modulations of aminergic systems in terms of transmitter availability through dispositional processes and compensatory receptor kinetic alterations, the principal functioning loci of antidepressant action still remain obscure. Thus, more fluid approaches such as cellular cybernetics at the transsynaptic level should be considered. Multiple actions of tricyclic antidepressants may result in both pre- and postsynaptic receptor sensitivity changes within the same (intra) neuronal tract or different (inter) neurons. This may be a plausible suggestion since interrelationships between central monoaminergic systems do exist [17].

Careful analysis of structure activity relationships have led to the introduction of selective aminergic uptake inhibitors. Compounds such as nisoxetine and maprotiline are considered to be selective NE uptake inhibitors [22] while CGP-6085 [43], nitalapram [43], fluoxetine [22], LM-5008 [24], and trazodone [27] are reportedly selective inhibitors of the reuptake of 5-HT. Preferential inhibition of multiple forms of MAO has also been addressed bringing forth compounds such as clorgyline and deprenyl [16]. Mianserine, appears to increase NE release by inhibiting presynaptic alpha-receptors [3] while another atypical non-tricyclic antidepressant, nomifensine [20] may operate through dopaminergic mechanisms, since disturbances in DA metabolism in endogenously depressed patients have been observed [5,41].

Therefore, if one assumes that the amine hypothesis of affective disorders allows for two types of depression, involving either serotonergic systems or catecholamine deficiencies, then selective modulations of aminergic systems through the use of these above-mentioned agents may prove to be reasonable therapeutic approaches in the management of subtypes of depressive illness.

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