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Effect of Antidepressant Drugs on Serotonergic and Adrenergic Receptors*,**

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Summary. We examined eight tricyclic antidepressants (doxepin, amitriptyline, clomipramine, imipramine, trimipramine, nortriptyline, desipramine and protriptyline) and four nontricyclic antidepressants (mianserin, nomifensin, iprindole and zimelidine) in terms of their effects on serotonergic and adrenergic receptors by direct binding assays. ^{3}H-WB-4101 (0.22 nM) served as a label for postsynaptic alpha receptors while ^{3}H-clonidine (0.2 nM) served to label presynaptic alpha receptors in the frontal cortex of the calf. ^{3}H-dihydroalprenolol (^{3}H-DHA) (0.5 nM) was used to label the beta$_1$ adrenoceptors in the calf frontal cortex and beta$_2$ adrenoceptors in the calf cerebellar cortex. ^{3}H-d-Lysergic acid diethylamide (^{3}H-LSD) (2 nM) and ^{3}H-serotonin (^{3}H-5HT) (0.5 nM) were used to label the serotonergic receptors in the calf frontal cortex.

The results show that at drug concentrations which occur clinically in the plasma water, most of the tertiary amine tricyclics significantly inhibited ^{3}H-WB-4101, ^{3}H-LSD and ^{3}H-5HT binding. None of the antidepressants tested had any significant effect on ^{3}H-DHA binding. The secondary amine tricyclics as a group were not potent in inhibiting the binding of all four radiolabelled ligands. The nontricyclic antidepressant mianserin was potent in inhibiting the binding of ^{3}H-WB-4101, ^{3}H-clonidine, ^{3}H-LSD and ^{3}H-5HT. We suggest that the differences between the effects of the antidepressants on adrenergic and serotonergic receptors may be responsible for the differences in their therapeutic efficacies and side effects.

Key words: Antidepressant drugs – Serotonin receptors – Adrenergic receptors – Plasma drug level.

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Introduction

Although tricyclic antidepressants are widely used in the treatment of depression, their mechanism of action remains unclear. The ability of some of these drugs to inhibit the neuronal reuptake of noradrenaline and serotonin (Glowinski and Axelrod, 1965; Carlsson et al., 1969a, 1969b) suggests that their effects on noradrenergic and serotonergic neurotransmission may be important for their clinical efficacies. As neurotransmission depends not only on the neurotransmitter itself but also on the neurotransmitter receptor, it is important to investigate the effect of tricyclic antidepressant drugs on adrenergic and serotonergic receptors. Radiolabelled ligands of high specific activity are available for the labelling of these receptors. In this study, we examined eight tricyclic antidepressants (doxepin, amitriptyline, clomipramine, imipramine, trimipramine, nortriptyline, desipramine and protriptyline) and four nontricyclic antidepressants (mianserin, nomifensin, maprotriline and zimelidine) in terms of their potencies in inhibiting the binding of two serotonergic (^{3}H-serotonin and d-^{3}H-lysergic acid diethylamide) and three adrenergic (^{3}H-WB-4101, ^{3}H-clonidine and ^{3}H-dihydroalprenolol) ligands to calf brain tissues.

Methods

Preparation of Calf Frontal and Cerebellar Homogenates. The procedure for preparing the tissues for radioreceptor assay has been described previously (Whitaker and Seeman, 1978a). Briefly, calf brains were obtained fresh from Canada Packers (Hunnisett plant, Toronto, Canada). The cortical gray matter was gently scraped from the frontal lobe and cerebellum and suspended in approximately 50 volumes of buffer (15 mM Tris HCl, pH 7.4, 5 mM Na_2 EDTA, 1.1 mM ascorbate and 12.5 µM nialamide). A preliminary crude homogenate of the suspension was made by using a glass homogenizer with a Teflon piston (0.13–0.18 mm clearance), rotated at 500 rpm, with 10 up and down strokes. After centrifugation at 39,000 g for 15 min at 4°, the supernatant was discarded and the pellet

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was resuspended in 20 volumes of buffer and homogenized with a Brinkmann Polytron at setting 7 for 10 s. This final preparation was then stored in vials at −20 until use. Before use, the membrane preparation was thawed, and homogenized for another 10 s with the Brinkmann Polytron at setting 7.

Radioreceptor Assays. The following [^{3}H] ligands were used: ^{3}H-WB-4101 [0.22 nM] for the post synaptic $\alpha_1$receptor (U'Prichard et al., 1977), ^{3}H-clonidine [0.2 nM] for the $\alpha_2$receptor (U'Prichard et al., 1977, 1978a, Titeler et al., 1978), ^{3}H-dihydroalprenolol (^{3}H-DHA) [0.5 nM] for the $\beta_1$receptor in calf frontal cortex and for the $\beta_2$receptor in calf cerebellar cortex, (U'Prichard et al., 1978b), ^{3}H-serotonin (^{3}H-5HT) [0.5 nM] and d-[^{3}H]-lysergic acid diethylamide (^{3}H-LSD) [2 nM] (in presence of 100 nM phentolamine) for serotonergic receptors (Whitaker and Seeman, 1978a, 1978b). Other studies have suggested that 5HT and LSD bind to similar, but not identical sites (Bennett and Snyder, 1976; Fillion et al., 1978). For ^{3}H-LSD binding, the 100 nM phentolamine was used to block adrenergic receptors present in the frontal cortex (Whitaker and Seeman, 1978b).

Triplicate incubation tubes each received 600 µl of [^{3}H] ligand, 300 µl of various concentrations of unlabelled drugs, 300 µl of buffer (composition as mentioned above) and 600 µl of tissue suspension. The total incubation volume was 1.8 ml.

Tubes were incubated at 21 for 30 min for ^{3}H-clonidine, ^{3}H-LSD and ^{3}H-5HT, 20 min for ^{3}H-DHA and 15 min for ^{3}H-WB-4101. Equilibrium conditions were reached for each ligand with the times of incubation employed. The contents were then rapidly filtered under vacuum through Whatman GF/B filters with 2 × 5 ml rinses of buffer. Filters were pre-wetted with 5 ml of buffer before filtration. The filters were then counted by liquid scintillation spectrometry in 10 ml of Aquasol (New England Nuclear) at an efficiency of 43 % after storage at 4° for at least 6 h.

Specific binding of ^{3}H-clonidine was defined as the excess over blanks taken in the presence of 1 µM clonidine. For ^{3}H-WB-4101 binding, it was defined as the amount of binding displaceable by 1 µM phentolamine. For ^{3}H-DHA binding, ^{3}H-LSD binding and ^{3}H-5HT binding, it was defined as the amount of binding displaceable by 10 µM (−) propranolol, 200 nM LSD and 100 nM 5HT, respectively.

^{3}H-Clonidine (26.7 Ci/mmol) was generously donated by Dr. Bechtel of Boehringer, Ingelheim, FRG. ^{3}H-WB-4101 (13 Ci/mmol), ^{3}H-DHA (51.1 Ci/mmol), ^{3}H-LSD (10.7 Ci/mmol) ^{3}H-5HT (28.2 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, Mass., USA). All ^{3}H-ligands were used without further purification.

Drugs. Antidepressant drugs used: Mianserin HCl (Organon, West Orange, New Jersey, USA), protriptyline HCl and amitryptyline HCl (Merck, Sharp & Dohme, Dorval, Quebec, Canada), clomipramine HCl, maprotiline HCl, imipramine HCl and desipramine HCl (Ciba-Geigy, Dorval, Quebec, Canada), doxepin HCl (Pfizer, Dorval, Quebec, Canada), trimipramine maleate (Poulenc, Montreal, Quebec, Canada), nortriptyline HCl (Eli Lilly, Toronto, Ontario, Canada), iprindole HCl (Wyeth, Windsor, Ontario, Canada), nomifensin (Hoechst, Montreal, Quebec, Canada), zimelidine, cis form (Astra Chemicals Pty. Ltd., Södertälje, Sweden).

Results

^{3}H-WB-4101 Binding

Significant potency was displayed by mianserin and some tertiary tricyclic antidepressants in the competition of ^{3}H-WB-4101 binding in the calf frontal cortex (Table 1, Fig. 1). The most potent agents included doxepin, amitriptyline, mianserin and trimipramine. The least potent agents were nomifensin, iprindole and zimelidine.

Table 1. Inhibition of ^{3}H-WB-4101 and ^{3}H-clonidine binding by antidepressant drugs. Values are means ± standard deviations of two to five separate experiments, each conducted in triplicate

Drug	IC_{50} values (nM)			
	^{3}H-WB-4101		^{3}H-clonidine	
	Calf frontal cortex	Rat whole brain[a] Excluding cerebellum	Calf frontal cortex	Rat whole brain[b] Excluding cerebellum
Tertiary amine tricyclics				
Doxepin	24 ± 6	32	2,000 ± 100	
Amitriptyline	46 ± 7	34	850 ± 250	896
Trimipramine	67 ± 6		1,700 ± 600	
Clomipramine	130 ± 35	77	7,430 ± 750	
Imipramine	160 ± 36	81	4,930 ± 1,200	4,901
Secondary amine tricyclics				
Nortriptyline	130 ± 27	99	2,980 ± 1,400	
Protriptyline	420 ± 95	388	10,360 ± 3,700	
Desipramine	440 ± 60	207	9,400 ± 4,300	
Other antidepressant drugs				
Mianserin	56 ± 16		12 ± 7	
Maprotiline	250 ± 80		16,770 ± 6,500	
Nomifensin	980 ± 370		2,480 ± 1,100	
Zimelidine	1,210 ± 460		610 ± 270	
Iprindole	6,150 ± 1,600		16,000 ± 1,400	

[a] Calculated from U'Prichard et al. (1978c)
[b] Calculated from U'Prichard et al. (1977)

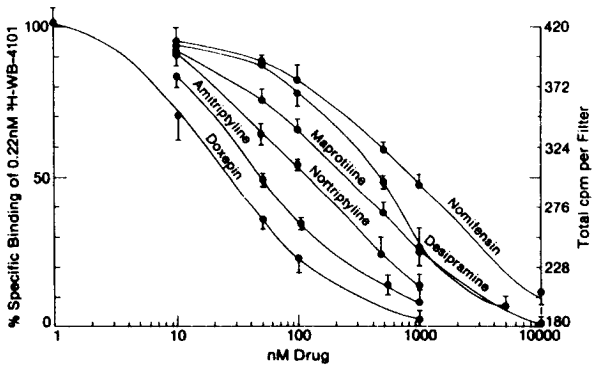

Fig. 1. The competition of antidepressant drugs against the binding of ^{3}H-WB-4101 to calf frontal cortex homogenates. Specific binding of ^{3}H-WB-4101 (0.22 nM) was defined as that displaceable by 1 μM phentolamine. Vertical bars indicate standard deviations

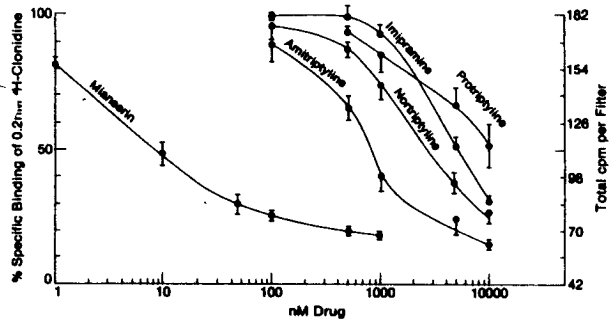

Fig. 2. The competition of antidepressant drugs against the specific binding of ^{3}H-clonidine to calf frontal cortex homogenates. Specific binding of ^{3}H-clonidine (0.2 nM) was defined as that displaceable by 1 μM clonidine. Vertical bars indicate standard deviations

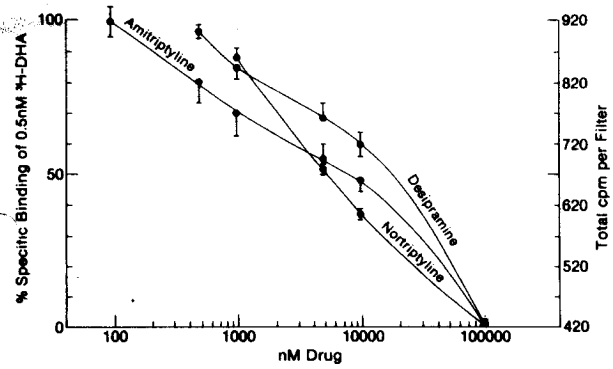

Fig. 3. The competition of tricyclic antidepressants against the binding of ^{3}H-DHA to calf frontal cortex homogenates. Specific binding of ^{3}H-DHA (0.5 nM) was defined as that displaceable by 1 μM (−) propranolol. Vertical bars indicate standard deviations

^{3}H-Clonidine Binding

Mianserin was the only antidepressant that significantly displaced ^{3}H-clonidine binding in the calf frontal cortex (Table 1, Fig. 2). In fact, mianserin was very potent in this aspect, having an IC_{50} of 12 nM.

Table 2. Inhibition of ^{3}H-dihydroalprenolol binding by antidepressant drugs

Drug	Calf	
	Frontal cortex	Cerebellar cortex
	IC_{50} values (nM)	
Tertiary amine tricyclics		
Trimipramine	5,300	13,000
Amitriptyline[b]	7,000	4,000
Clomipramine	9,300	21,000
Imipramine	13,300	13,500
Doxepin	20,500	7,600
Secondary amine tricyclics		
Nortriptyline	5,400	3,600
Protriptyline	8,700	10,000
Desipramine[a]	14,200	3,000
Other antidepressant drugs		
Maprotiline	7,900	5,400
Zimelidine	8,500	36,000
Mianserin	11,200	37,000
Iprindole	17,000	9,600
Nomifensin	23,300	66,000

[a] IC_{50} of desipramine was over 100,000 nM on rat cardiac membrane preparation (Harden et al., 1976) and 7,560 nM (calculated from Maguire et al., 1976) on cultured rat glioma cells for ^{125}I-hydroxybenzylpindolol (^{125}I-HYP) binding

[b] IC_{50} of amitriptyline was 32,400 nM for ^{125}I-HYP binding on cultured rat glioma cells (calculated from Maguire et al., 1976)

^{3}H-DHA Binding

None of the antidepressants tested were potent in displacing ^{3}H-DHA binding either in the calf frontal cortex (Table 2, Fig. 3) or cerebellum (Table 2).

^{3}H-Serotonin and ^{3}H-LSD Binding

Several antidepressants had weak but significant inhibition of the binding of these two ligands. Mianserin was the most potent antidepressant in this aspect (Table 3, Figs. 4, 5).

Discussion

While the results showed that all the antidepressants tested had little effect on ^{3}H-DHA and ^{3}H-clonidine binding (except mianserin), their inhibitory effects on the binding of the other three ligands differed remarkably. For example, iprindole had an IC_{50} (concentration for 50% inhibition) of 6150 nM for ^{3}H-WB-4101 binding, more than two hundred times that of doxepin (24 nM). For both ^{3}H-LSD and ^{3}H-5HT

Table 3. Competition of antidepressant drugs against the binding of ^{3}H-5HT and ^{3}H-LSD to calf frontal cortex homogenates. Values are means ± standard deviations of two to five separate experiments, each conducted in triplicate

Drug	IC_{50} (nM)	
	^{3}H-Serotonin	^{3}H-LSD
Tertiary amine tricyclics		
Doxepin	240 ± 20	190 ± 60
Amitriptyline	240 ± 40	140 ± 36
Trimipramine	320 ± 130	300 ± 60
Clomipramine	590 ± 210	410 ± 180
Imipramine	1,080 ± 490	560 ± 270
Secondary amine tricyclics		
Nortriptyline	380 ± 30	350 ± 120
Protriptyline	1,150 ± 150	900 ± 250
Desipramine	3,070 ± 1,400	2,000 ± 580
Other antidepressants		
Mianserin	90 ± 30	100 ± 28
Maprotiline	220 ± 18	1,000 ± 150
Nomifensin	1,370 ± 230	2,700 ± 630
Zimelidine	2,500 ± 1,250	4,400 ± 1,000
Iprindole	6,000 ± 1,700	5,800 ± 200

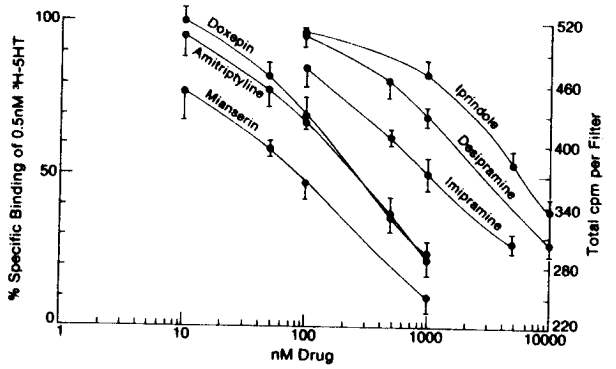

Fig. 4. The competition of antidepressant drugs against the binding of ^{3}H-5HT to calf frontal cortex homogenates. Specific binding of ^{3}H-5HT (0.5 nM) was defined as that displaceable by 100 nM 5HT. Vertical bars indicate standard deviations

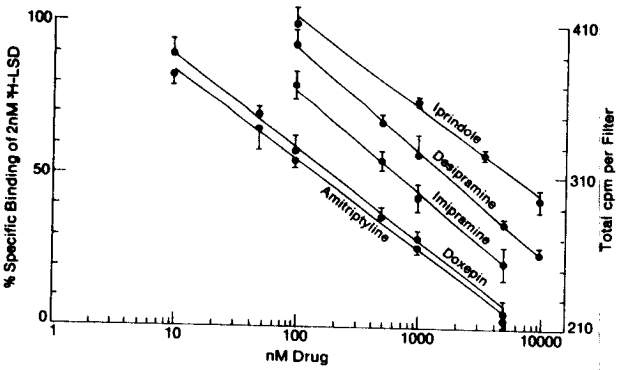

Fig. 5. The competition of antidepressant drugs against the binding of ^{3}H-LSD to calf frontal cortex homogenates in the presence of 100 nM phentolamine. Specific bindings of ^{3}H-LSD (2 nM) was defined as that displaceable by 200 nM LSD. Vertical bars indicate standard deviations

binding, the IC_{50} of mianserin (100 nM) was about sixty times less than that of iprindole (6000 nM).

Allowing for 90% plasma protein binding, the free plasma drug concentrations for these antidepressants and their active metabolites would be below 200 nM in most patients (Cooper et al., 1976; Gram et al., 1975). An examination of the IC_{50}'s of the thirteen antidepressants on ^{3}H-WB-4101 binding shows that these drugs could be roughly grouped into two groups. One group, represented by mianserin, nortriptyline, amitriptyline, and other tertiary amine tricyclics, was effective in inhibiting ^{3}H-WB-4101 binding at relatively low concentrations (IC_{50} below 200 nM). The other group, represented by desipramine, protriptyline and other non-tricyclic antidepressant drugs, was only effective at high concentrations (IC_{50} above 200 nM). It has been suggested that the effects of the antidepressant drugs on the ^{3}H-WB-4101 binding sites represent blockade of functional α-adrenergic receptors (U'Prichard et al., 1978c). Thus, the first group of antidepressant drugs may not produce any enhancement of noradrenaline responses at the α-adrenergic receptors, even though they themselves or their metabolites may potently inhibit noradrenaline reuptake. This same view has been earlier expressed by Iversen (1975).

For ^{3}H-5HT and ^{3}H-LSD binding, only three drugs (mianserin, doxepin and amitriptyline) have low IC_{50} values. Some antidepressant drugs have been shown to be antagonists of serotonin receptors (Domenjoz and Theobald, 1959; Fuxe et al., 1977; Sigg, 1959; Saxena et al., 1971). The effects of the antidepressant drugs on the ^{3}H-5HT and ^{3}H-LSD binding sites may represent blockade of serotonin receptors. Potentiation of serotonergic transmission thus may not occur when the plasma drug level becomes high enough to cause a blockade of these receptors, even though some of these drugs are potent serotonin reuptake inhibitors. How this is related to the therapeutic window demonstrated for some tricyclic antidepressants (Yates et al., 1963; Kragh-Sorenson, 1976; Whyte et al., 1976; Montgomery et al., 1977; Friedel et al., 1979) would be an interesting area to explore.

There is also a high correlation between the IC_{50}'s for ^{3}H-WB-4101 binding and ^{3}H-LSD binding (Fig. 6). This appears to be in accord with the suggestion that serotonergic receptors and α-adrenoceptors, although

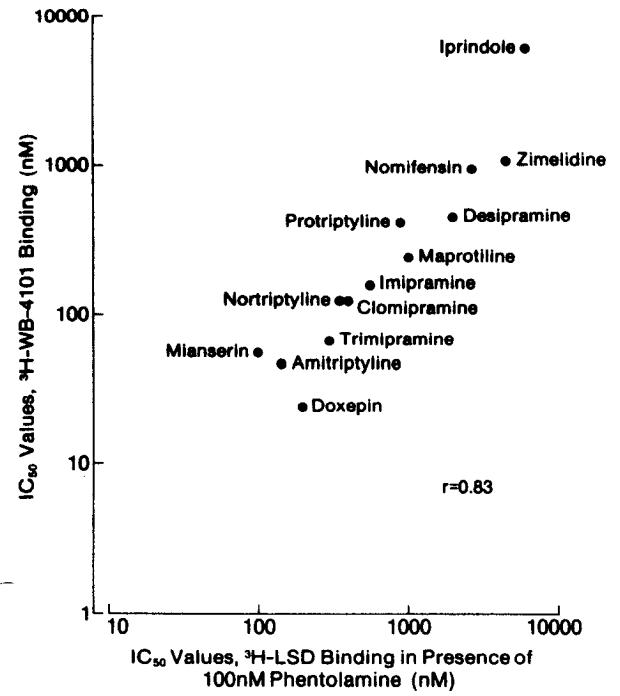

Fig. 6. Correlation between the IC_{50} values for various antidepressant drugs against the binding of ^{3}H-WB-4101 and ^{3}H-LSD (in presence 100 nM phentolamine) in calf frontal cortex homogenates

distinct entities, have features in common (Apperley et al., 1976; Kapur and Mottram, 1979).

The displacement of ^{3}H-clonidine binding by mianserin probably represents a blockade of pre-synaptic α-adrenergic receptors. ^{3}H-clonidine labels an α-adrenergic receptor which has the property of a pre-synaptic α-receptor (U'Prichard et al., 1978a; Titeler et al., 1978). Other studies also suggested that mianserin blocks the pre-synaptic α-adrenergic receptors (Bauman and Maitre, 1977; Tang et al., 1979). Thus, although mianserin is a weak noradrenaline reuptake inhibitor (Goodlet et al., 1977), it can result in an increase in synaptic noradrenaline concentration by increasing noradrenaline release.

In this experiment, we only studied the effects of antidepressant drugs on serotonergic and adrenergic receptors. This is because that an increased availability of noradrenaline and/or serotonin at post-synaptic sites has been generally accepted as part of the therapeutic action of such drugs. Many clinical studies also support the hypothesis of altered noradrenaline and/or serotonin metabolism in depressed patients (Ashcroft et al., 1966; Denker et al., 1966; van Praag et al., 1970; Coppen et al., 1972; Maas et al., 1972; Mendels et al., 1972; Schildkraut, 1973; Goodwin and Beckmann, 1975; Garfinkel et al., 1977). Our results show that the antidepressant drugs are not a uniform class of drugs in terms of their action on serotonergic and adrenergic receptors. One has to note, of course, that the chronic effects of antidepressant drugs on these receptors could be very different from their acute ones. For example, development of β-receptor subsensitivity after long-term treatment with some antidepressants has been reported by several groups (Banerjee et al., 1977; Sarai et al., 1978; Clements-Jewery, 1978; Rosenblatt et al., 1979; Bergstrom et al., 1979).

Antidepressant drugs also posses potent activities on many other receptors. Many of these drugs have been shown to be antagonists of muscarinic receptors (Domenjoz and Theobald, 1959; Sigg, 1959; Rehavi et al., 1977; Snyder and Yamamura, 1977; Rehavi and Sokolovsky, 1978), histamine H_1 receptors (Domenjoz and Theobald, 1959; Sigg, 1959; Psychoyos, 1978; Richelson, 1978, 1979) and histamine H_2 receptors (Green and Maayani, 1977; Kanof and Greengard, 1978; Rehavi and Sokolovsky, 1978). Recently, a high-affinity binding site for ^{3}H-imipramine in rat cerebral cortex has also been reported (Raisman et al., 1979). For these other receptor sites, the antidepressant drugs again show no uniformity in their actions.

It is thus likely that antidepressant drugs may not have a common mechanism of action. On the other hand, depression is not a homogeneous illness (Maas, 1978) and different antidepressant drugs may be required for different subtypes of depression (Maas et al., 1972, 1975; Schildkraut, 1973).

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