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Reevaluation of the Indoleamine Hypothesis of Depression. Evidence for a Reduction of Functional Activity of Central 5-HT Systems by Antidepressant Drugs

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With 1 Figure

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Summary

The effects of antidepressant drugs on central 5-HT receptor activity were studied in rats and mice. Antidepressant drugs were evaluated for their ability to displace ^{3}H-5-HT and ^{3}H-d-LSD from membrane binding sites in the dorsal neocortex of rats *in vitro* and for their ability to block 5-HTP and d-LSD induced behavioral effects in mice. The degree of blockade of head-twitches in mice produced by the antidepressants was highly correlated with their affinity for ^{3}H-d-LSD binding sites. A number of antidepressant drugs such as amitriptyline, nortriptyline, mianserine, doxepine, nomifensine and dibenzepine appear to possess marked 5-HT receptor blocking activity at some types of 5-HT receptors in brain. New antidepressant drugs such as zimelidine, which specifically inhibit 5-HT reuptake and do not block 5-HT receptor sites, may after chronic treatment also reduce the functional activity of 5-HT systems by producing adaptive changes in postsynaptic 5-HT mechanisms. Thus, a new indoleamine hypothesis of depression is presented: the therapeutic action of antidepressant drugs may in part be due to a reduced functional activity of some central 5-HT systems.

Introduction

The amine hypothesis of depression postulates that depression is due to reduced neuronal transmission at noradrenaline (NA) (*Schildkraut*, 1965; *Bunney et al.*, 1965) or 5-hydroxytryptamine (5-HT)

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(*Coppen*, 1967; *Lapin* and *Oxenkrug*, 1969; *van Praag*, 1974) synapses in the brain. The early observations that tricyclic antidepressants inhibited the neuronal uptake of NA (*Glowinski* and *Axelrod*, 1964; *Iversen*, 1965; *Carlsson et al.*, 1966) and the subsequent discovery that some of them also inhibited the uptake of 5-HT (*Ross* and *Renyi*, 1969; *Carlsson et al.*, 1969) suggested that the therapeutic action of antidepressants was due to an increased availability of NA or 5-HT at postsynaptic receptor sites (*Schildkraut*, 1965; *Bunney et al.*, 1965; *Coppen*, 1967; *Lapin* and *Oxenkrug*, 1969; *van Praag*, 1974).

However, several findings are difficult to reconcile with this view. Some antidepressants such as iprindole, doxepine and mianserine appear to be very weak inhibitors of NA and 5-HT uptake *in vivo* (*Ross et al.*, 1971; *Leonard*, 1974). In addition, two of the most commonly used tricyclic antidepressants, amitriptyline and nortriptyline were recently found to have a high affinity for ^{3}H-d-LSD binding sites in the brain (*Fuxe et al.*, 1977, 1978 a) and also reduced central 5-HT activity in a behavioral test. Previous studies have shown that imipramine, desipramine, chlorimipramine and mianserine can reduce specific high affinity ^{3}H-d-LSD binding in the rat brain (*Bennett* and *Aghajanian*, 1975; *Bennett* and *Snyder*, 1976). Behavioral studies in the rat have also indicated that mianserine and doxepine are blockers of postsynaptic 5-HT receptors (*Maj et al.*, 1978).

In the present paper we have examined the 5-HT receptor blocking properties of a number of tricyclic antidepressants having different chemical structures including newly introduced putative antidepressants of a nontricyclic type. The ability of the tested drugs to displace specifically bound ^{3}H-5-HT and ^{3}H-d-LSD and to block a behavioral response (head-twitches) induced by 5-hydroxytryptophan and d-LSD was examined and the results from the two types of experiments were correlated.

Material and Methods

Binding Experiments

Male Sprague-Dawley rats (150—200 g) and male albino mice (NMRI strain, 18—22 g) were used. The studies on specific ^{3}H-5-HT and ^{3}H-d-LSD binding were performed *in vitro* in the dorsal neocortex of rats. The procedure was the same as that described by *Bennett* and *Snyder* (1976). Specific binding was defined as the difference between the total counts in the absence of unlabelled ligand and the counts obtained in presence of

unlabelled d-LSD (1 μM) or unlabelled 5-HT (10 μM). Saturation analysis of ^{3}H-d-LSD binding revealed that the dissociation constants and the maximal number of binding sites for ^{3}H-5-HT (K_D = 10 nM; B_{max} = 18 pmoles/g net weight) and ^{3}H-d-LSD (K_D = 6 nM; B_{max} = 39 pmoles/g net weight) were similar to those previously described (*Bennett* and *Snyder*, 1976; *Fuxe et al.*, 1977). IC_{50}, based on 5—6 concentrations, was obtained from Hill plots. K_i was calculated according to the formula

$$K_i = \frac{IC_{50}}{1 + \frac{C}{K_D}}.$$

C = concentration of the radioactive ligand, K_D = dissociation constant. The Hill coefficients were also obtained from the Hill plot of the displacement curves and the 95 % confidence intervals of the Hill coefficient were calculated according to Sen's procedure, a nonparametric method (*Theil*, 1950; *Sen*, 1968). Each experiment was repeated 2—3 times and five replicates were made at each concentration. The concentration of ^{3}H-5-HT (16—17 Ci/mmol) was 5—6 nM and that of ^{3}H-d-LSD (11.4 Ci per mmol) 2—3 nM. ^{3}H-5-HT and ^{3}H-d-LSD were purchased from New England Nuclear and from Amersham-Searle respectively.

Behavioral Experiments

Studies on 5-HT receptor activity *in vivo* were performed in mice. The 5-HT precursor 5-hydroxytryptophan (5-HTP) and the 5-HT agonists d-LSD induce head-twitches in mice which appear to be due to increased 5-HT receptor activity (*Corne et al.*, 1963; *Ögren* and *Ross*, 1977; *Nakamura* and *Fukushima*, 1978). Potentiation of 5-HTP induced head-twitches was performed as described previously (*Ögren* and *Ross*, 1977). The drugs were injected i.p. 60 min before dl-5-HTP. Blockade of the 5-HTP and d-LSD induced head-twitches were performed in analogy with the potentiation of 5-HTP (*Fuxe et al.*, 1977). The drugs were injected i.p. 60 min before 5-HTP and d-LSD. In these experiments a strong behavioral response was used as a base line to minimize the possibility of an unspecific blockade. Therefore, 5-HTP (90 mg/kg i.v.) was given to animals pretreated with the monoamine oxidase inhibitor 1-α-ethyltryptamine (5 mg/kg i.p., 60 min before the 5-HTP injection) and a relatively high dose of d-LSD (0.5 mg/kg s.c.) was used. This procedure produces a high frequency of head-twitches. The specificity of the 5-HTP and d-LSD blockade was further assessed by testing the blockade of the pinna reflex (*Corne et al.*, 1963) 60 min after the i.p. injection of the drugs. ED_{50}'s were calculated by probit analysis (*Finney*, 1952) based on 5—6 dose levels with 10 animals per dose. The Spearman Rank test (*Conover*, 1971) was used to test for correlations. Similarities between the tested drugs were analyzed by means of a dendrogram based on Sokal and Sneath's procedure (*Sokal* and *Sneath*, 1963).

Compounds

The drugs were dissolved in physiological saline or in distilled water and injected i.p. in a volume of 10 ml/kg. The following clinically used antidepressant drugs representing various chemical structures were tested:

Dibenzazepines: imipramine HCl (Ciba-Geigy), desipramine HCl (Ciba-Geigy) and chlorimipramine HCl (Ciba-Geigy); dibenzocycloheptadienes: amitriptyline HCl (Lundbeck), nortriptyline HCl (Ciba-Geigy) and protriptyline HCl (Merck); dibenzoxepine: doxepine HCl (Pfizer); N-substituted cycloalkylindole: iprindole HCl (Wyeth): tetrahydroisoquinoline: nomifensine maleate (Hoechst) (*Hoffman*, 1973); dibenzodiazepine: dibenzepine HCl (Hässle) (*Collard et al.*, 1973); tetracyclic piperazineazepine: mianserine HCl (Organon) (*Vargaftig et al.*, 1971). Some new types of putative antidepressants were also included, *i.e.* zimelidine [(Z)-3-(4-bromophenyl)-N,N-dimethyl-3-(3-pyridyl)-allylamine dihydrochloride monohydrate] (Astra) and fluoxetine [3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine HCl] (Eli Lilly) which are both potent and selective inhibitors of 5-HT uptake *in vivo* (*Ross et al.*, 1976; *Wong et al.*, 1975). Zimelidine has recently been reported to have an antidepressant action in man (*Coppen et al.*, 1979; *Montgomery et al.*, 1978). The 5-HT receptor blocking agent methergoline (Farmitalia) (*Fuxe et al.*, 1975) and the neuroleptic drug chlorpromazine HCl (Leo) were included for comparison.

Results

Binding Studies

The results are summarized in Table 1. Calculated correlations are shown in Table 2. The drugs studied have been listed according to their potency to displace specific ^{3}H-d-LSD binding in the dorsal neocortex of the rat. The antidepressants and 5-HT but not d-LSD or methergoline, displaced d-LSD binding with a Hill coefficient significantly different from 1 ranging from 0.16 to 0.71. Doxepine (0.56) and nomifensine (0.71) had the highest Hill coefficients while chlorimipramine (0.16) had the lowest. The K_i values showed that amitriptyline, nortriptyline, mianserine and doxepine had a substantial affinity for specific ^{3}H-d-LSD binding (K_i's ranging from 48—550 nM) while dibenzepine, protriptyline, iprindol, fluoxetine and zimelidine had a low affinity (K_i's > 3000 nM). Desipramine, nomifensine and imipramine were moderately active with K_i's ranging from 710—1500 nM. The neuroleptic agent chlorpromazine was potent in displacing ^{3}H-d-LSD ($K_i = 110$ nM). Many antidepressants such as nortriptyline, mianserine, doxepine and dibenzepine also displaced ^{3}H-5-HT binding with a Hill coeffi-

cient significantly different from 1. Imipramine (0.94) and chlorimipramine (0.72) however, had a Hill coefficient similar to that obtained with 5-HT (0.77). Amitriptyline, nortriptyline, chlorpromazine, mianserine, desipramine, nomifensine and doxepine (K_i values ranging from 500—1260 nM) had a lower affinity for the ^{3}H-5-HT binding site than for the ^{3}H-d-LSD binding site. Imipramine, chlorimipramine, iprindol and protriptyline on the other hand had a much higher affinity for the ^{3}H-5-HT binding sites (K_i values ranging from 120—490 nM) than for the ^{3}H-d-LSD binding sites. Fluoxetine and zimelidine had a low affinity for the ^{3}H-5-HT binding site also. Specific 5-HT and d-LSD binding was only slightly affected by the adrenergic blocking agent phenoxybenzamine (IC_{50} = 3000 nM), the histamine H_2-receptor antagonist cimetidine (IC_{50} ~ 3000 nM) and the DA receptor antagonist haloperidol (IC_{50} = 2000 nM). No correlation between the K_i values of the tested drugs for displacement of ^{3}H-d-LSD and ^{3}H-5-HT binding (r_s = —0.11, n.s.) was found (Table 2). However, when considering only those drugs which preferentially displaced ^{3}H-5-HT, there was a significant correlation between K_i values for the displacement of ^{3}H-d-LSD and ^{3}H-5-HT binding (r_s = 0.95, $p < 0.05$).

Behavioral Studies

All the drugs which had a substantial affinity for the d-LSD binding site were found to block the head-twitches induced by d-LSD and 5-HTP in mice. In agreement with the binding results, amitriptyline, nortriptyline, chlorpromazine, mianserine, doxepine, nomifensine and dibenzepine were the most active in blocking the actions of 5-HTP and d-LSD. Chlorimipramine and imipramine were moderately active and desipramine and protriptyline had a low potency in blocking 5-HTP and d-LSD. Zimelidine, iprindol and fluoxetine failed to block 5-HTP and d-LSD even in high doses ($ED_{50} > 110$ μmol/kg). Most tested compounds did not block the pinna reflex in mice in doses up to 150 μmol/kg i.p. with the exception of amitriptyline (ED_{50} = 90 μmol/kg i.p.) and nortriptyline (ED_{50} = 120 μmol/kg i.p.).

Blockade of d-LSD and 5-HTP induced head-twitches were highly correlated (r_s = 0.96, $p < 0.001$) (Table 2). There was also a highly significant correlation between the K_i values for displacement of d-LSD binding and the ED_{50} values for the inhibition of d-LSD (r_s = 0.78, $p < 0.005$) and 5-HTP induced head-twitches (r_s = 0.83, $p < 0.001$). Such a correlation was not found between the affinity of the tested drugs for the 5-HT binding site and their ability to block

Table 1. *Behavioral and biochemical studies on the effects of antidepressant drugs on postsynaptic 5-HT receptors in the rat. Displacement of ^{3}H-d-LSD and ^{3}H-5-HT from membrane binding sites in the cortex cerebri of rats, blockade of d-LSD and 5-HTP induced head-twitches and potentiation of 5-HTP induced head-twitches in mice*

Drug	^{3}H-d-LSD[a] K_i, nM	Hill coeff. (95 % conf. limit)	d-LSD[b] Head-twitches blockade ED_{50} (S.E.M.) μmol/kg i.p.	^{3}H-5-HT[a] K_i, nM	Hill coeff. (95 % conf. limit)	5-HTP[b] Head-twitches blockade ED_{50} (S.E.M.) μmol/kg i.p.	5-HTP[c] Head-twitches Potentiation ED_{50} (S.E.M.) μmol/kg i.p.	Clinical[d] dose μmol/kg/day
5-HT	126	0.43 (0.16)	—	5.6	0.77 (0.45)	—		—
d-LSD	2.9	0.91 (0.21)	—	12	0.47 (0.49)	—		—
Methergoline	10	0.89 (0.09)	0.5 (0.09)	62	0.55 (0.48)	0.13 (0.02)	>10	—
Amitriptyline[e]	48	0.39 (0.16)	7.5 (1.6)	1260	0.47 (0.59)	15 (1.8)	>80	8.5
Nortriptyline[e]	66	0.30 (0.22)	20 (3.0)	1200	0.35 (0.44)	30 (5.0)	>80	6
Chlorpromazine	110	0.48 (0.61)	4.2 (0.6)	690	0.14 (0.51)	5.9 (0.8)	>20	5
Mianserine	190	0.31 (0.33)	6.6 (1.1)	500	0.35 (0.51)	18 (4.0)	>80	2
Doxepine	550	0.56 (0.42)	24 (4.4)	720	0.47 (0.15)	22 (3.8)	>80	10.5
Desipramine	710	0.39 (0.09)	103 (10.6)	>3000	—	>110	>80	8
Nomifensine	1000	0.71 (0.32)	13 (2.1)	>10000	—	30 (5.4)	>80	7
Imipramine	1500	0.35 (0.13)	81 (6.9)	150	0.94 (0.49)	56 (7.6)	34.4 (8.2)	7.5
Chlorimipramine	2100	0.16 (0.16)	62 (9.1)	190	0.72 (0.50)	53 (6.8)	7.7 (1.6)	5
Dibenzepine	4400	0.40 (0.36)	41 (6.0)	320	0.23 (0.16)	56 (11.8)	>80	18.5
Protriptyline	5000	0.43 (0.13)	95 (9.0)	490	0.41 (0.50)	128 (7.0)	>80	2
Zimelidine	>3000	—	>110	>3000	—	>110	8.6 (1.7)	6
Iprindol	19000	0.35 (0.66)	>110	430	0.41 (0.52)	>110	>80	5
Fluoxetine	23000	0.32 (0.42)	>110	2000	0.18 (0.24)	>110	5.2 (0.98)	—

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Dibenzepine	)0	0.40 (0.36)	41 (6.0)	320	0.23)6)	56 (11.8)	>80	18.5
Protriptyline	5000	0.43 (0.13)	95 (9.0)	490	0.41 (0.50)	128 (7.0)	>80	2
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Iprindol	19000	0.35 (0.66)	>110	430	0.41 (0.52)	>110	>80	5
Fluoxetine	23000	0.32 (0.42)	>110	2000	0.18 (0.24)	>110	5.2 (0.98)	—

[a] The binding procedure was performed according to the method of *Bennett* and *Snyder* (1976). Each experiment was repeated 2—3 times and 5—7 concentrations were tested with 5 replicates. The Hill coefficient and IC_{50} was calculated from Hill plots and 95% confidence intervals around the Hill coefficient was calculated according to Sen's procedure. $K_i = \frac{IC_{50}}{1 + \frac{C}{K_D}}$

C = concentration of the radioactive ligand,
K_D = dissociation constant.

[b] Blockade of d-LSD (0.5 mg/kg s.c.) and 5-HTP (90 mg/kg i.v.) induced head-twitches were performed in mice. The compounds were injected i.p. 60 min prior to d-LSD or 5-HTP. The monoamine oxidase inhibitor 1-α-ethyltryptamine (5 mg/kg i.p.) was injected 60 min before the 5-HTP injection. ED_{50} based on 5—6 dose levels with 10 animals per dose level was calculated by probit analysis.

[c] Potentiation of 5-HTP (90 mg/kg i.v.) induced head-twitches was conducted in mice. The compounds were injected i.p. 60 min prior to 5-HTP. ED_{50} based on 5—6 dose levels with 10 animals per dose level was calculated by probit analysis.

[d] The average clinical doses are standard doses for treatment of depression as recommended by the manufacturers (see also *Klein* and *Davis*, 1969) and they are based on a body weight of 70 kg.

[e] The results taken from *Fuxe et al.* (1977).

Table 2. *Correlation matrix of the effects of antidepressant drugs on some 5-HT and NA mechanisms and on improvement of depression in man*

		Binding		Blockade of head-twitches		Inhibition of uptake			
						in vitro		*in vivo*	
		^{3}H-d-LSD	^{3}H-5-HT	d-LSD	5-HTP	5-HT	NA	5-HT[a]	NA
Binding	^{3}H-d-LSD	—							
	^{3}H-5-HT	—0.11 (n.s.)	—						
Blockade of head-twitches	d-LSD	0.78***	0.17 (n.s.)	—					
	5-HTP	0.83***	0.10 (n.s.)	0.96***	—				
Inhibition of uptake *in vitro*	5-HT	—0.24 (n.s.)	—0.15 (n.s.)	—0.43 (n.s.)	—0.32 (n.s.)	—			
	NA	0.43 (n.s.)	0.17 (n.s.)	—0.54 (n.s.)	—0.74**	—0.14 (n.s.)			
Clinical dose		—0.13 (n.s.)	0.12 (n.s.)	0.20 (n.s.)	0.17 (n.s.)	—0.28 (n.s.)	0.20 (n.s.)		0.27 (n.s.)

The experimental results of Table 1 and literature values have been transformed into rank values. The values of the effects of the tested drugs on the uptake of NA and 5-HT *in vitro* are based on previous studies using crude synaptosomes prepared from the hypothalamic or forebrain regions of the rat (see *Buus Lassen et al.*, 1975; *Koe*, 1976; *Placheta et al.*, 1976; *Randrup* and *Braestrup*, 1977; *Ross et al.*, 1973; *Ross* and *Renyi*, 1975, 1977; *Wong et al.*, 1975). The values of the drugs on the uptake of 5-HT and NA *in vivo* are based on previous studies performed in mice (see *Carnmalm et al.*, 1975; *Ross* and *Renyi*, 1967, 1969; *Ross et al.*, 1971, 1976; *Ross*, 1979). The inhibition of the uptake of 5-HT and NA was measured in the cortex cerebri or in the midbrain regions of mice 30—60 min after i.p. administration. The ranking was based on K_i values, ED_{50}'s or EC_{50}'s. EC_{50} refers to the concentration and ED_{50} to the dose which inhibits active uptake by 50%. The statistical correlation between the parameters was calculated by the Spearman Rank correlation Test (*Conover*, 1971). *** $p < 0.005$, ** $p < 0.025$.

[a] Not possible to calculate due to insufficient data.

d-LSD (r_s = 0.17, n.s.) and 5-HTP induced head-twitches (r_s = 0.10, n.s.). When considering only those drugs which preferentially affected 5-HT binding, the correlation between the K_i values for the displacement of ^{3}H-5-HT and the ability to block d-LSD and 5-HTP induced head-twitches was 0.72 (n.s.) and 0.78 (n.s.) respectively. In agreement with the binding results, most antidepressants were more potent in antagonizing d-LSD than 5-HTP induced head-twitches.

The behavioral and biochemical results obtained in this study and results derived from the existing literature on the effects of the tested drugs on NA and 5-HT uptake did not correlate with the clinical doses used for treatment of depression (Table 2). There was also no significant correlation between the effects of the tested drugs on NA

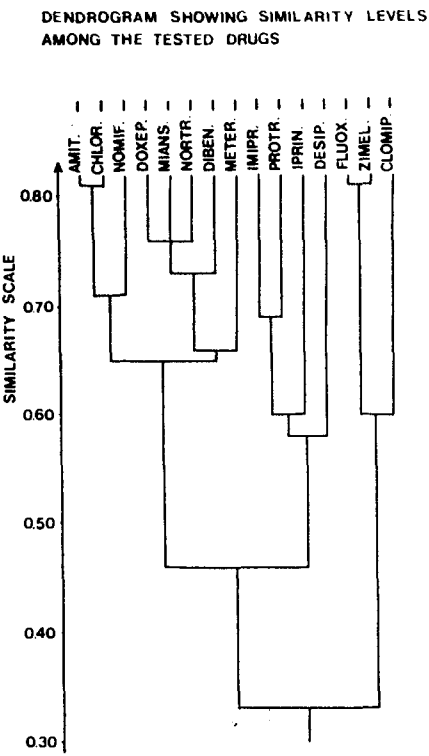

Fig. 1. The similarity between different types of antidepressant drugs shown by a dendrogram. The dendrogram was made according to the procedure of *Sokal* and *Sneath* (1963). The dendrogram is based on the results in Tables 1 and 2. The calculated similarity levels between the tested drugs and between the clusters of drugs are shown on the similarity scale. According to this analysis the tested drugs can be grouped in four groups (see Results)

and 5-HT and effects on ^{3}H-5-HT and ^{3}H-d-LSD binding. The results were further analyzed by means of a similarity matrix for the drugs studied (not shown) based on the parameters shown in Tables 1 and 2. The dendrogram made according to the procedure of *Sokal* and *Sneath* summarizes the results (Fig. 1). According to this analysis, the overall similarity level of the antidepressant drugs is low (0.33, n.s.) and the antidepressants can be grouped as follows (similarity levels within brachets).

Group 1. This group consists of amitriptyline and chlorpromazine (0.81) joined by nomifensine at a similarity level of 0.71.

Group 2. Doxepine, mianserine and nortriptyline (0.76) are joined by dibenzepine at a similarity level of 0.73. The first and the second group form a single cluster at a similarity level of 0.65.

Group 3. Imipramine and protriptyline (0.69) are joined by iprindol and desipramine at a similarity level of 0.60 and 0.58 respectively. The first three groups form a single cluster at a similarity level of 0.46.

Group 4. Zimelidine and fluoxetine (0.81) are joined by chlorimipramine at a similarity level of 0.60.

Discussion

The radioligands ^{3}H-5-HT and ^{3}H-d-LSD have been used by several groups to evaluate actions of drugs at central postsynaptic 5-HT receptors (*Bennett* and *Aghajanian*, 1975; *Bennett* and *Snyder*, 1976; *Lovell* and *Freedman*, 1976; *Fuxe et al.*, 1977, 1978 a; *Bourgoin et al.*, 1978; *Fillion et al.*, 1978). These studies, based on lesions of 5-HT pathways (*Fuxe et al.*, 1978 b; *Azmitia*, 1978) and on studies with 5-HT receptor blocking agents (*Bennett* and *Aghajanian*, 1975; *Bennett* and *Snyder*, 1976; *Lovell* and *Freedman*, 1976; *Bourgoin et al.*, 1978; *Fuxe et al.*, 1978 b), favor the view that ^{3}H-5-HT and ^{3}H-d-LSD binding sites represent separate sites (see *Furchgott*, 1978) and that at least some of the ^{3}H-d-LSD binding sites are linked to the postsynaptic 5-HT receptors. It must be emphasized again that d-LSD pharmacologically mimics 5-HT in several functional and electrophysiological models (*Andén et al.*, 1968; *Haigler* and *Aghajanian*, 1974) and seems to be a mixed agonist-antagonist at postsynaptic 5-HT receptor sites (*Bennett* and *Snyder*, 1976; *Aghajanian* and *Wang*, 1978). Behavioral evidence also indicates that d-LSD acts

upon central 5-HT receptors. A number of studies suggest that head-twitches induced by d-LSD and high doses of 5-HTP are due to activation of postsynaptic 5-HT receptors (*Corne et al.*, 1963; *Jacobs*, 1976; *Ögren* and *Ross*, 1977; *Nakamura* and *Fukushima*, 1978). Furthermore, following destruction of central 5-HT terminals by 5, 7-dihydroxytryptamine, the behavioral responses to 5-HT precursors and d-LSD are markedly potentiated (*Trulson et al.*, 1976). The specificity of the head-twitch response for 5-HT receptors is further underlined by studies with different receptor blockers. Thus, the DA-receptor blocking agent haloperidol, the α-adrenergic blockers phenoxybenzamine and the histamine H_2-receptor blocking agent cimetidine reduced head-twitches induced by 5-HTP and d-LSD in a very high dose range indicating a non-specific action (unpublished findings). In contrast, drugs such as methergoline which block central 5-HT receptors (*Fuxe et al.*, 1975; *Fuxe et al.*, 1978 c) antagonize in very low doses head-twitches induced by d-LSD and 5-HTP (*Fuxe et al.*, 1978 c) while zimelidine and other inhibitors of the neuronal uptake of 5-HT potentiate head-twitches induced by 5-HTP (*Ross et al.*, 1976).

The present findings taken together indicate that many of the most commonly used antidepressant drugs reduce central 5-HT synaptic activity by blocking certain types of 5-HT receptors in the brain. Moreover, the blockade of the head-twitch response to d-LSD or 5-HTP appears not to be due to a non-specific action in view of the relatively weak blockade of the pinnareflex by the antidepressants. Amitriptyline, nortriptyline, mianserine, doxepine, desipramine and nomifensine belong to this group of antidepressant drugs which show a higher affinity for the d-LSD than for the 5-HT binding site in the cerebral cortex. The observation that these drugs, unlike d-LSD and methergoline, displace ^{3}H-d-LSD binding with a Hill coefficient significantly lower than 1 can be explained on the basis that they only bind to some of the d-LSD binding sites. Thus, both 5-HT receptor antagonists and 5-HT agonists (*Bennett* and *Snyder*, 1976; *Fuxe et al.*, 1977) can displace ^{3}H-d-LSD binding with a low Hill coefficient (cf. *Burt et al.*, 1976). The behavioral studies show that these antidepressant drugs do not potentiate head-twitches induced by 5-HTP. On the contrary, they block 5-HTP and d-LSD induced head-twitches. The rank order of this blockade is highly correlated to the ability to displace ^{3}H-d-LSD from binding sites. Thus, the mechanism for the blockade of the 5-HTP and d-LSD syndrome observed following acute treatment with some antidepressants may partly be due to occupation of some regulatory d-LSD binding sites related to the 5-HT receptors (see *Aghajanian* and *Wang*, 1978).

Certain antidepressants such as imipramine, chlorimipramine, dibenzepine, iprindol and protriptyline primarily displaced ^{3}H-5-HT binding in the cerebral cortex and with varying Hill coefficients. The behavioral studies show that dibenzepine did not potentiate but blocked 5-HTP and d-LSD induced head-twitches in relatively low doses. Imipramine and chlorimipramine on the other hand potentiated 5-HTP in lower doses and blocked 5-HTP and d-LSD induced head-twitches in higher doses. Thus, both the behavioral and biochemical results suggest that imipramine and chlorimipramine have both a presynaptic (5-HT uptake blockade) and postsynaptic action (blockade of some postsynaptic 5-HT receptors). There is a positive though non-significant correlation between the ability of these drugs to block 5-HTP and d-LSD induced head-twitches and to displace ^{3}H-5-HT binding. The trend from the correlation analyses suggest that these drugs, too, may block the head-twitches induced by d-LSD and 5-HTP via blockade of some 5-HT receptors. It should be pointed out that the K_i values of these drugs for displacement of ^{3}H-5-HT and ^{3}H-d-LSD binding exhibit a significant correlation. Note that imipramine and chlorimipramine also have a relatively high Hill coefficient (0.72 to 0.94), thus mimicking 5-HT in this respect.

The high affinity of iprindol for the ^{3}H-5-HT binding sites in the cortex was not correlated with any effect on the head-twitch response in mice. Head-twitch behavior, however, appears to be induced by actions on postsynaptic 5-HT mechanisms in the brain stem (*Jacobs*, 1976). A functional model for studies on postsynaptic cortical 5-HT receptor mechanisms is not available. It is possible, therefore, that the observed discrepancy is due to a selective action of iprindol on cortical 5-HT receptors.

In contrast to most antidepressants, the two putative antidepressants zimelidine and fluoxetine were shown to potentiate 5-HTP in doses well related to their ability to inhibit 5-HT uptake without any signs of blockade of the head-twitches induced by 5-HTP and d-LSD. Thus, both these drugs appear to mainly have a presynaptic action (*i.e.* inhibition of 5-HT uptake).

In view of these novel findings the indoleamine hypothesis of depression may be modified. Thus, in certain depressive states 5-HT synaptic mechanisms may be relatively overactive (*Shaw et al.*, 1977; *Fuxe et al.*, 1977; *Fuxe et al.*, 1978 a) and not as previously suggested underactive (see Introduction). A similar hypothesis has recently been put forward by *Aprison* and collaborators based *inter alia* on studies on animal behavior indicating that increased release of 5-HT results in a behaviorally depressive state (*Aprison et al.*, 1978). Such a hypothesis is also in line with the present knowledge on the role of

5-HT in anxiety, negative reinforcement and punishment (*Stein et al.*, 1975; *Aghajanian* and *Wang*, 1978) and the behavioral and neuroendocrinological disturbances observed in depression (*Carrol* and *Mendels*, 1976; *Shaw et al.*, 1977).

When comparing the 5-HT receptor blocking action of the tested drugs with their ability to inhibit 5-HT and NA-uptake *in vivo* it is found that certain types of antidepressants such as amitriptyline, nortriptyline, mianserine, dibenzepine, nomifensine and doxepine possess 5-HT receptor blocking activity in doses near to or below those causing 5-HT and NA uptake inhibition. The K_i values for displacement of d-LSD and 5-HT binding could be of therapeutic significance since the K_i values are below the concentration range found in the rat brain after injection of tricyclic antidepressants (*Nagy*, 1977) and mianserine (*Kafoe et al.*, 1976) in rats. The K_i values are also in the same order of magnitude as the concentration values of antidepressants such as amitriptyline present in the plasma of patients (*Coppen et al.*, 1978). The observation that chlorpromazine appears to block some 5-HT receptor sites (low Hill coefficient) does not necessarily contradict the present hypothesis. Although it is not clear whether chlorpromazine is a specific antidepressant, some authors have reported that chlorpromazine in low doses may show antidepressant properties (*Paykel et al.*, 1968). The fact that no significant correlation between any behavioral or biochemical effects measured in this study and clinical potencies were found cannot be taken as a strong argument against the present hypothesis in a view of the high variability in clinical dosage and the complex metabolic and pharmacokinetic behavior of these drugs in man.

There is a seemingly paradoxical situation in that certain antidepressants such as amitriptyline, nortriptyline, mianserine etc. acutely block central 5-HT receptors, whereas others such as zimelidine enhance central 5-HT activity. This apparent paradox may be resolved by studying the chronic effects of such antidepressants on central 5-HT mechanisms. It was recently demonstrated that administration of the 5-HT uptake inhibitor zimelidine for a period of 14 days induced a low affinity binding site with a high number of binding sites for 5-HT in the cerebral cortex of rats (*Fuxe et al.*, 1979). This finding indicates that chronic 5-HT uptake inhibition may induce adaptive changes in the postsynaptic 5-HT mechanisms. Furthermore, the reduction of 5-HT synthesis observed after acute treatment with zimelidine (*Carlsson* and *Lindqvist*, 1978) was not attenuated following long-term treatment (*Fuxe et al.*, 1979). It is therefore possible that in the chronic treatment situation with zimelidine adaptive changes in postsynaptic 5-HT mechanisms combined with a reduced

5-HT release could lead to a reduced postsynaptic 5-HT receptor activity. It therefore seems likely that the 5-HT reuptake blockade may be of less importance during chronic treatment. Moreover, it may be speculated that the acute inhibition of firing rate in 5-HT neurons (cf. *Aghajanian*, 1972) exist also following chronic treatment with drugs preferentially inhibiting 5-HT uptake, an action which may be important for their antidepressant properties. In a similar way the ability of tryptophan to enhance the antidepressant action of chlorimipramine (*Wålinder et al.*, 1975) could be related to a further enhancement of the reduction of firing rate in 5-HT neurons produced by chlorimipramine (*Aghajanian*, 1972). Such mechanisms could in part also explain the antidepressant activity of monoamine oxidase inhibitors.

In view of the present findings it also seems possible to explain the recent finding that long-term treatment with several tricyclic antidepressants increase responsivity of rat forebrain neurons to 5-HT (*de Montigny* and *Aghajanian*, 1978). Such changes in postsynaptic 5-HT sensitivity could be related to the 5-HT receptor blocking activity of certain antidepressants as well as to their ability to reduce impulse flow in 5-HT neurons and 5-HT release (cf. *Aghajanian* and *Wang*, 1978). It is also tempting to speculate that the latency of about 3 weeks for development of antidepressant activity in man after treatment of several types of antidepressant drugs could in part be related to the induction of adaptive changes in 5-HT mechanisms. In this context it should be pointed out that desipramine and protriptyline were found to be weak 5-HT blocking agent while potently blocking NA uptake. However, recent findings with desipramine indicates that this drug can increase 5-HT utilization in very low doses in the cortex cerebri (unpublished findings). Therefore, these two drugs may also have important actions on 5-HT mechanisms. Taken together, the most prominent actions of antidepressant drugs are either to inhibit NA reuptake and/or to affect 5-HT synaptic mechanisms by blocking some 5-HT receptors or via 5-HT uptake inhibition produce a down regulation of 5-HT receptor activity. Thus, an important requirement for antidepressant action may be to alter the ratio of NA receptor activity to 5-HT receptor activity in the brain.

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