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A Possible Role of Serotonin Receptors in Antidepressant Drug Action

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Summary

Recently research into the mechanism of action of antidepressant drugs has focused on mechanisms that could account for the delayed onset of therapeutic action. Chronic treatment with antidepressants elicits a delayed reduction in numbers of beta-adrenergic receptor binding sites as well as norepinephrine sensitive adenylate cyclase. Besides catecholamines, serotonin mechanisms have been thought to play a major role in the actions of antidepressants. We have characterized two discrete serotonin receptors using ligand binding techniques. S_1-receptors are labelled selectively by ^{3}H-serotonin and S_2-receptors bind ^{3}H-spiroperidol, while ^{3}H-LSD has equal affinities for S_1 and S_2 receptors. Relative potencies of drugs in competing for S_1-receptors show some correlations with effects on adenylate cyclase. Taken together with the regulation of S_1-receptors by guanine nucleotides, a characteristic of adenylate cyclase linked receptors, we suggested, that S_1-receptors may have some association with adenylate cyclase.

S_2-receptors, on the other hand, are not regulated by guanine nucleotides. Relative potencies of drugs in competing for S_2-receptors parallel closely their ability to block serotonin related behavioral syndromes elicited by a variety of drugs. Thus the behavioral effects of most serotonin related drugs appear to be by S_2-receptors.

Chronic but not acute administration of both tricyclic antidepressants and monoamineoxidase inhibitors lowers the numbers of S_2-receptors. For most drugs these effects are more pronounced than the reduction in numbers of beta-adrenergic receptors. This effect is selective, since neuroleptics such as chlorpromazine and haloperidol are ineffective. Only a few antidepressants reduce numbers of S_1-receptors and these effects are lesser than the reduction in S_2-receptors. By contrast chronic treatment with antidepressants fails to alter numbers of dopamine, alpha-adrenergic or muscarinic cholinergic receptors. Thus, it appears likely that reduction in numbers of S_2-serotonin receptors as well as beta-adrenergic receptors are related to the antidepressant actions of drugs.

Mögliche Beteiligung der Serotoninrezeptoren am Wirkungsmechanismus der Antidepressiva

Kürzliche Untersuchungen über den Wirkungsmechanismus der Antidepressiva galten den Mechanismen, die den verzögerten Eintritt der therapeutischen Wirkung erklären könnten. Langzeitbehandlung und Antidepressiva verlangsamt die Verminderung der Anzahl der beta-adrenergen Rezeptoren und der noradrenalinempfindlichen Adenylatzyklase. Neben den Katecholaminen hat man den Serotoninmechanismen eine maßgebliche Beteiligung am Wirkungsmechanismus der Antidepressiva zugeschrieben. Zwei verschiedene Arten von Serotoninrezeptoren lassen sich unter Anwendung von Liganden-Bindungstechniken charakterisieren. S_1-Rezeptoren werden selektiv mit ^{3}H-Serotonin markiert und S_2-Rezeptoren binden ^{3}H-Spiroperidol, während ^{3}H-LSD gleiche Affinitäten für S_1- und S_2-Rezeptoren besitzt. Die relativen Wirksamkeiten von Pharmaka im Wettbewerb um die S_1-Rezeptoren zeigen einige Korrelationen mit der Wirkung auf Adenylatzyklase. Unter Berücksichtigung der Tatsache, daß S_1-Rezeptoren durch Guanin-Nukleotide beeinflußt werden, welches eine charakteristische Eigenschaft von Rezeptoren darstellt, die eine Affinität zu Adenylatzyklase besitzen, vermuteten wir eine mögliche Beeinflussung der S_1-Rezeptoren durch Adenylatzyklase. S_2-Rezeptoren werden dagegen nicht durch Guanin-Nukleotide beeinflußt. Die relativen Wirksamkeiten von Pharmaka im Wettbewerb um S_2-Rezeptoren laufen stark mit deren Fähigkeiten parallel, Verhaltenssyndrome zu blockieren, die zu der Wirkung des Serotonins in Beziehung stehen und durch eine Vielzahl von Pharmaka hervorgerufen werden. Daher scheinen die durch die meisten mit dem Serotonin in einem Zusammenhang stehenden Pharmaka ausgelösten Wirkungen auf Verhaltensweisen durch S_2-Rezeptoren vermittelt zu werden.

Eine Langzeitverabreichung, nicht jedoch eine kurzfristige Verabreichung, sowohl trizyklischer Antidepressiva als auch von Monoaminooxidasehemmern vermindert die Anzahl der S_2-Rezeptoren. Bei den meisten antidepressiven Pharmaka sind diese Wirkungen ausgeprägter als die Verringerung der Anzahl der beta-adrenergen Rezeptoren. Diese Wirkung hat selektiven Charakter, da Psychopharmaka wie z.B. Chlorpromazin und Haloperidol keine derartige Wirkung ausüben. Nur wenige Antidepressiva vermindern die Anzahl der S_1-Rezeptoren, und diese Wirkung fällt weniger ins Gewicht als die Verringerung der Anzahl der S_2-Rezeptoren. Im Gegensatz hierzu verändert eine Langzeitbehandlung mit Antidepressiva nicht die Anzahl der Dopamin-, alpha-adrenergen oder muskarinartigen cholingergen Rezeptoren. Es erscheint daher wahrscheinlich, daß eine Verminderung der Anzahl sowohl der S_2-Serotoninrezeptoren als auch der beta-adrenergen Rezeptoren mit der antidepressiven Wirkung bestimmter Pharmaka in einem Zusammenhang stehen.

Since their first introduction into clinical medicine antidepressants have been linked to the monoamines, norepinephrine and serotonin. The monoamine oxidase inhibitors elevate brain levels of these two amines while tricyclic antidepressants inhibit their reuptake inactivation. Accordingly, one could merely postulate that their antidepressant effects involve facilitating actions of the monoamines one way or another. The relatively recent development of clinical effective antidepressants, such as iprindole, which do not inhibit amine uptake, posed problems for this simple formulation. Another troubling aspect was that these effects on biogenic amines occur acutely, while the clinical antidepressant effects of the drugs require treatment for periods of ten days to two weeks of more. Accordingly, researchers began seeking biological markers affected by chronic but not acute treatment with these drugs.

These dilemmas seemed to be solved rather neatly by the finding of Sulser and associates that chronic treatment

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with antidepressants with various classes reduces the activity of a norepinephrine sensitive adenylate cyclase in brain homogenates (9). The enzyme being monitored in these studies (9) is not explicitly alpha or beta in character. However, regardless of the exact nature of the receptor involved, its activity is reduced following chronic treatment even with atypical antipressant such as iprindole. Electroconvulsive shock treatment also lowers the activity of the norepinephrine sensitive adenylate cyclase.

Monitoring adrenergic receptors by binding techniques has shown that beta adrenergic receptors are reduced in number following chronic treatment with various antidepressants including monoamine oxidase inhibitors, tricyclic antidepressants and the atypical antidepressant drugs (1).

Our interest in this area came with observations of two distinct serotonin receptors that can be differentially labeled by binding techniques (6). Our earlier work had involved labeling serotonin receptors with ^{3}H-LSD or ^{3}H-serotonin (2). The drug specificity of binding sites labeled by the two ligands was somewhat different. However, we initially attributed this to the fact that LSD was a mixed agonist-antagonist while serotonin was a pure agonist. The subsequent use of ^{3}H-spiroperidol to label serotonin receptors clarified the discrepancies. ^{3}H-Serotonin and ^{3}H-spiroperidol indeed bind to distinct populations of serotonin receptors in the brain, while ^{3}H-LSD binds to both types with similar affinity (6). This explains why drug potencies at ^{3}H-LSD binding sites are intermediate between affinities for the ^{3}H-serotonin and the ^{3}H-spiroperidol sites. The receptors that bind serotonin, designated S_1 receptors, are regulated by guanine nucleotides, whereas sites that bind spiroperidol (S_2) are not influenced by the nucleotides (4).

We were able to obtain evidence for differential physiological functions associated with the two distinct serotonin receptors (5) (Fig. 1). The potencies of drugs in inhibiting a serotonin sensitive adenylate cyclase correlated with their potencies at the ^{3}H-serotonin labeled, hence S_1, receptors though the complexities of the serotonin sensitive adenylase cyclase precluded strong conclusions about the identity of the binding sites with the receptor linked cyclase. A more unequivocal relationship was found between affinities of drugs for ^{3}H-spiroperidol labeled S_2 receptors and their potencies in blocking behavioral effects referred to as the "serotonin syndrome". A well characterized syndrome of behavioral hyperactivity follows central serotonin stimulation with drugs such 5-hydroxytryptophan, tryptophan plus monoamine oxidase inhibitors, LSD and quipazine. Potencies of drugs in blocking these behavioral effects correlate closely with their affinities for S_2 receptors indicating that the S_2 receptors mediate these behavioral actions. Indeed, it seems that all reported biological functions influenced by known serotonin antagonists involve S_2 receptors. Except for a possible relationship to the serotonin sensitive cyclase, the S_1 receptor is a receptor "in search of a function". Interestingly, this resembles the situation with dopamine receptors in which the D_1 receptor linked to the dopamine sensitive adenylate cyclase does not correspond to any known physiological event.

To understand the behavioral functions of the two serotonin receptors, we evaluated the effects of chronic treatment with a variety of drugs, especially antidepressants and neuroleptics. To ascertain specificity of any observed responses, we measured binding associated with a variety of receptors including histamine H_1, muscarinic cholinergic,

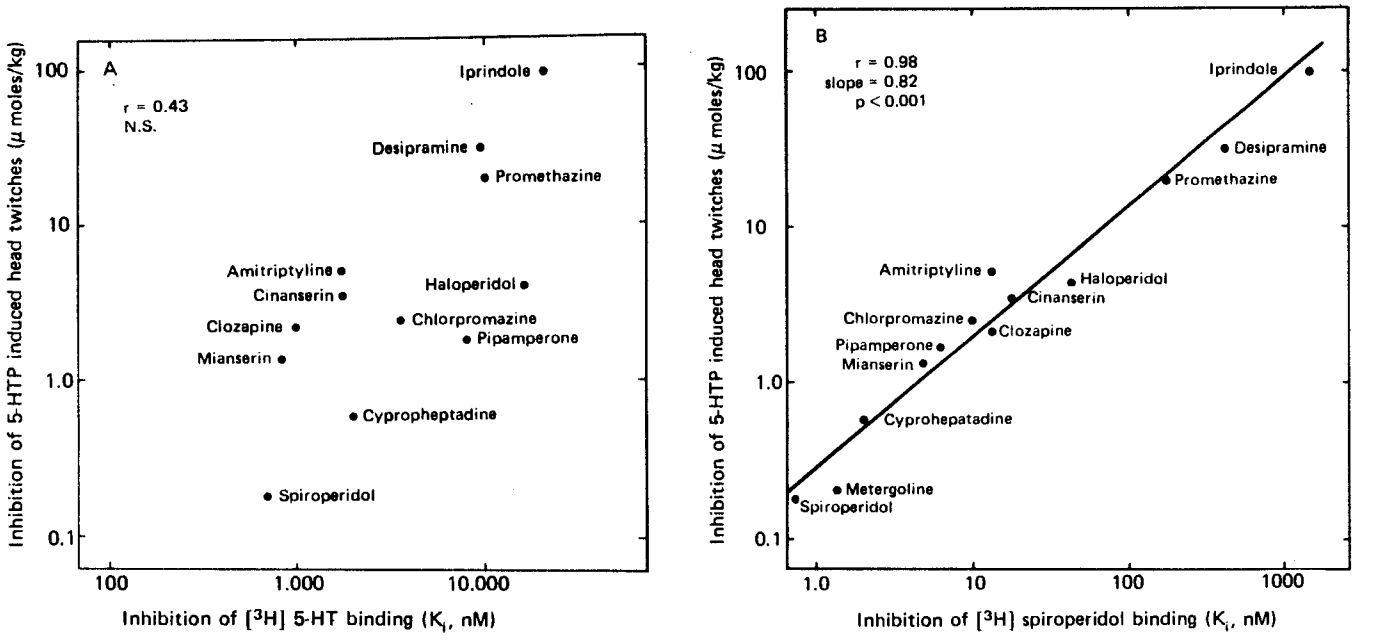

Fig. 1 Comparison of drug affinities at two serotonin receptors with drug inhibition of serotonin-related behavior. Behavioral studies were performed on mice after the injection of 5-hydroxytryptophan 30 minutes before a 2-minute observation period. Various drugs were injected 1 hour before the observation period. The number of head twitches were recorded at four or five concentrations, and ID_{50} values were calculated for log-probit analysis. Slopes of these plots were parallel for all drugs. Standard errors did not exceed 15 percent of mean values. Data were statistically compared by linear regression analysis

Table 1 Effect of long-term drug treatment on neurotransmitter receptor binding in rat brain. The relative amount of ^{3}H-labeled ligand bound (saline control = 100) was determined in homogenates of rat brain after 21 consecutive days of treatment with equivalent volumes of drugs or saline. Each value is the mean ± standard error from three to six separate animals. The experiments were replicated twice. The concentrations of the ligands were 0.36 nM [^{3}H]spiroperidol, 3.7 nM [^{3}H]LSD, 2.0 nM [^{3}H]serotonin, 0.8 nM [^{3}H]DHA, 0.2 nM [^{3}H]QNB, and 0.9 nM [^{3}H]WB-4101. Specific binding was defined as the excess over blanks taken in the presence of 1 μM d-LSD for serotonin receptors, 1 μM (±)-propranolol for β-adrenergic receptors, 0.1 mM oxotremorine for muscarinic cholingergic receptors, 0.1 mM (−)-norepinephrine for α-adrenergic receptors, and 1 μM (+)-butaclamol for dopamine receptors. All experiments were performed in triplicate. Student's t-test was used for statistical analysis of the raw data before conversion to percentages

Drug	Serotonin-2 (^{3}H-spiroperidol)	Serotonin-1$^+$ serotonin-2 (^{3}H-LSD)	Serotonin-1 (^{3}H-serotonin)	β-Adrenergic (^{3}H-DHA)	Muscarinic cholinergic (^{3}H-QNB)	α-Adrenergic (^{3}H-WB-4101)	Dopamine [^{3}H]spiroperidol
			Tricyclic antidepressants				
Amitriptyline	57 ± 3.0*	80 ± 1.7*	90 ± 3.7	82 ± 3.9$^+$	103 ± 2.6	97 ± 6.8	101 ± 4.5
Imipramine	60 ± 4.5*	74 ± 7.0$^+$	80 ± 3.5$^+$	82 ± 3.5$^+$	100 ± 0.72	104 ± 6.1	96 ± 4.4
Desipramine	79 ± 4.1*	82 ± 5.2$^+$	94 ± 2.6	71 ± 6.2$^+$	105 ± 1.9	97 ± 8.8	97 ± 4.2
			Atypical antidepressants				
Iprindole	66 ± 4.1*	79 ± 6.6$^+$	92 ± 6.4	88 ± 1.9$^+$	95 ± 2.2	107 ± 3.8	100 ± 2.8
Fluoxitene	87 ± 6.3	92 ± 9.7	98 ± 3.8	97 ± 5.8	97 ± 2.1	102 ± 4.3	99 ± 4.0
			MAO inhibitor				
Pargyline	65 ± 5.8*	65 ± 0.65*	58 ± 2.7*	85 ± 3.1$^+$	101 ± 0.54	97 ± 2.9	104 ± 1.5
			Serotonin antagonist				
Methysergide	90 ± 7.2	95 ± 1.2	102 ± 1.3	98 ± 3.9	104 ± 6.3	100 ± 2.6	100 ± 6.0
			Neuroleptics				
Chlorpromazine	90 ± 4.7	90 ± 4.4	105 ± 7.4	100 ± 4.2	100 ± 2.8	102 ± 1.5	132 ± 2.0*
Haloperidol	98 ± 6.1	102 ± 4.5	93 ± 3.2	102 ± 5.4	102 ± 0.79	101 ± 2.7	139 ± 6.4*

*$P < .01$; $^+P < .05$

alpha adrenergic, beta adrenergic and dopamine receptors as well as S_1 and S_2 serotonin receptors (7, 8).

We confirmed the finding that long-term treatment with the tricyclic antidepressants, the atypical antidepressant iprindole and the monoamine oxidase inhibitor pargyline reduces numbers of beta adrenergic receptors (Table 1). A substantially greater reduction of ^{3}H-spiroperidol binding to S_2 receptors occurs after long-term treatment with the antidepressants. Amitriptyline and imipramine reduce beta-adrenergic binding 20% but S_2 binding by 40%. Iprindole and pargyline also reduce binding to S_2 receptors more than to beta adrenergic receptors. Desipramine is the only antidepressant tested wherein the decline in beta receptors exceeds that for S_2 receptors. Fluoxitene, a potent inhibitor of serotonin but not of norepinephrine uptake, does not affect numbers of either beta adrenergic or S_2 receptors. Interestingly, in general its clinical antidepressant effects are equivocal.

Numbers of S_1 serotonin receptors also decline with chronic administration of imipramine and pargyline, but not with any of the other antidepressants, suggesting that effects on S_1 receptors are less related to antidepressant efficacy. Consistent with its labeling of both S_1 and S_2 receptors, ^{3}H-LSD binding is affected by long-term treatment with antidepressants in a fashion intermediate to effects on ^{3}H-spiroperidol and ^{3}H-serotonin binding.

Long-term drug treatment has no effect on muscarinic cholinergic, alpha adrenergic or dopamine receptor binding, emphasizing the specific nature of actions on β-adrenergic and S_2 receptors.

Scatchard analyses indicate that the reduction in serotonin receptor binding involves a change in numbers of sites with no alteration in affinity and cannot be accounted for by residual drug in the brain (8).

Most interestingly, the time course of alterations in numbers of S_2 receptors fits with what would be expected if the drug effects are related to therapeutic actions. Thus, while there may be some decrease in both S_2 and beta adrenergic receptors one week following amitriptyline, maximal reduction requires three weeks (Fig. 2) (8).

How might these findings fit with other data on the pharmacology of depression? Depletion of monoamines by reserpine is associated with clinical depression. The tricyclic antidepressant blockade of uptake systems and the inhibition of monoamine oxidase by drugs both enhance amine activity. Iprindole is considered an atypical antidepressant because of its failure to block uptake and inhibit monoamine oxidase. It presumably potentiates central biogenic amines, since it does increase central effects of amphetamine.

The simplest way of condeptualizing the chronic effects of antidepressants on adrenergic and serotonin receptors is to assume that it represents down regulation of the receptors in response to the enhancement of amine activity. This formulation has the advantage of integrating most of the known facts about drugs and affective disturbances.

There are some problems with this interpretation. Presumably down regulation of receptors implies subsensitivity. However, *Aghajanian* and colleagues have found enhanced

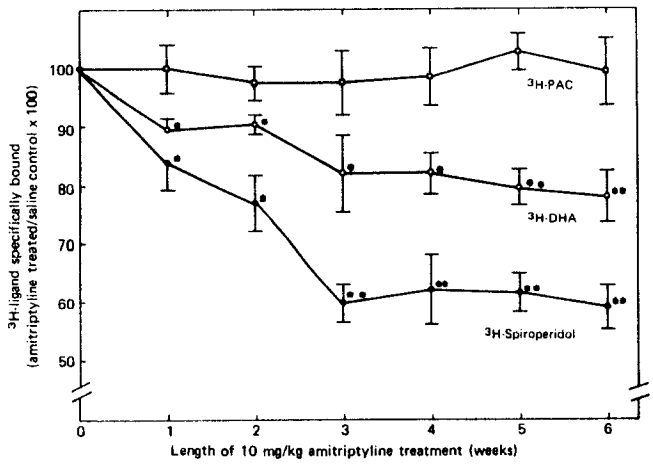

Fig. 2 Effect of amitriptyline administration on sertonin S_2 and adrenergic receptor binding in rat cerebral cortex. The amount of [^{3}H]spiroperidol, [^{3}H]DHA and [^{3}H]PAC specifically bound was determined in rat brain homogenates at weekly intervals after the initiation of daily 10 mg/kg of amitriptyline or 0.9 percent NaCl treatment. Each point is the mean ± S.E. of from three to seven animals from two different experiments and represents the percentage of saline control values. The Student's t-test was used to analyze the raw data. Differences were noted at $p < .05$ (*) and $p < .01$ (**) levels as indicated for each data point. Mean specific [^{3}H]spiroperidol, [^{3}H]DHA and [^{3}H]PAC bound in control samples was 800, 900 and 720 cpm, respectively

sensitivity both to norepinephrine at alpha receptors and to serotonin at identified synapses in the brain following chronic antidepressant treatment (3, 10). There may be a simple explanation for the discrepancy between the reduced numbers of receptors implying subsensitivity and the physiological supersensitivity. In the studies of *Aghajanian*, the enhancement of 5-HT effects was noted one day after the discontinuation of treatment. Two or more days after terminating drug treatment the enhanced receptor activity began to disappear and in some cases subsensitivity was observed (3, 10). Thus, the *in vivo* neurophysiologic studies might in fact correlate with the binding studies after the antidepressants have been washed out of the membranes. In other words, perhaps the enhanced sensitivity observed by *Aghajanian* was associated with continued presence of the antidepressant influencing synaptic events with subsensitive receptors becoming apparent after the drug leaves the body.

In summary, the reduction in number of serotonin S_2 and β-adrenergic receptors following antidepressant drug treatment presently represents the best "common final pathway" correlating with therapeutic response to all forms of antidepressant drug treatment. These effects provide a valuable tool for screening candidate antidepressant drugs. Additionally, they may point toward a common site of action of the drugs and thence to pathophysiologic alterations associated with clinical depression.

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