

Platelet α_2 -Adrenergic Receptors in Schizophrenic Patients Before and After Phenothiazine Treatment

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Abstract. The specific binding to isolated platelet membranes of ^3H -clonidine, an α_2 -adrenergic receptor partial agonist, and ^3H -yohimbine, an α_2 -adrenergic receptor antagonist, was measured in male, drug-free schizophrenic patients. The maximum number of binding sites (B_{max}) for ^3H -yohimbine was significantly lower in these patients than in normal subjects. Treatment with chlorpromazine (CPZ) for 2 weeks further decreased the B_{max} for both ligands. Plasma catecholamine levels were determined before and after treatment. Before treatment, levels of dopamine and norepinephrine (NE) were within a normal range, while epinephrine (E) levels were significantly elevated. CPZ treatment significantly increased plasma NE levels, but decreased E levels to a normal range. These observations suggest that schizophrenia might be associated with abnormal noradrenergic function that is reflected by a decreased number of platelet α_2 -adrenergic receptors.

Key Words. α_2 -Adrenergic receptor, chlorpromazine, human blood platelets, norepinephrine, schizophrenia.

Increasing evidence suggests that abnormal noradrenergic neuronal or receptor function, or both, may be involved in schizophrenia (Hornykiewicz, 1982). While antipsychotic medications are thought to have their primary therapeutic effects through blockade of dopamine receptors (Bunney, 1974; van Kammen, 1979), these drugs have potent α -adrenergic blocking activity as well (Janssen and Van Bever, 1978). Post-mortem studies of catecholamine content of various brain areas of schizophrenic patients show increased amounts of norepinephrine (NE) in the limbic forebrain (Farley et al., 1978), corpus striatum (Crow et al., 1979), and brainstem (Carlsson, 1979). Sternberg et al. (1982) found that clonidine decreases plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) content less in schizophrenic patients than in normal subjects, a finding that suggests a functional subsensitivity of α_2 -adrenergic receptors in such patients.

The α_2 -adrenergic receptor that is located on nerve endings is an autoreceptor which regulates the release of NE (Langer, 1977; Starke, 1977, 1981). The α_2 -adrenergic receptor is also located on other tissues, one of which is the human blood platelet (Garcia-Sevilla et al., 1981a). Through the use of receptor binding techniques, the

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α -adrenergic receptor on human platelets was shown to be of the α_2 -subtype and was found to be similar in its pharmacological and biochemical characteristics to those found in the rat brain (Garcia-Sevilla et al., 1981a; Smith et al., 1981). Abnormal numbers of platelet α_2 -adrenergic receptors have been observed in a variety of psychiatric disorders, which include panic-anxiety disorder (Cameron et al., 1983) and major depressive disorder (Garcia-Sevilla et al., 1981b; Smith et al., 1983). Recently, an increased number of α -adrenergic receptors on blood platelets has been reported for schizophrenic patients (Kafka and van Kammen, 1983).

The purpose of the present study was to assess further the possible existence of an α_2 -receptor abnormality in schizophrenic patients and to determine the effect of chlorpromazine (CPZ) treatment. The specific binding of ^3H -clonidine, an α_2 -adrenergic receptor partial agonist, and ^3H -yohimbine, an α_2 -adrenergic receptor antagonist, was used to determine the maximum number of binding sites (B_{max}) and dissociation constants (K_d) for the two ligands. In addition, plasma levels of NE, dopamine (DA), and epinephrine (E) were assessed before and after treatment to determine whether the number of platelet α_2 -adrenergic receptors was correlated with circulating catecholamine levels.

The present study shows that in male, drug-free schizophrenic patients, the number of platelet α_2 -adrenergic receptors as measured by ^3H -yohimbine binding is decreased compared to normal subjects. CPZ treatment further decreased the number of α_2 -adrenergic receptors as measured by the specific binding of either ^3H -clonidine or ^3H -yohimbine. Treatment with CPZ also significantly elevated plasma NE levels.

Methods

Diagnostic Procedure. All participants in the study were chronic schizophrenic male inpatients at the Ann Arbor Veterans Administration Medical Center. Each patient was interviewed by a senior attending psychiatrist and was included in the study if he met Research Diagnostic Criteria (Spitzer et al., 1978) for schizophrenia.

Patients were kept drug free for a period of at least 2 weeks before the study. Several patients were drug free for 1 year or more. Control subjects were drug-free, age-matched male volunteers who had no history of any psychiatric or nonpsychiatric disorder.

Of a total of 13 patients who initially consented to participate in the study, three completed the study but were later eliminated from the results because a diagnosis of primary affective disorder could not be ruled out. Of the 10 remaining patients, 9 were diagnosed with subtypes of paranoid (2), hebephrenic (1), or undifferentiated schizophrenia (6). One patient was diagnosed as having schizoaffective disorder. All patients were administered the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), Beck Depression Inventory (BDI) (Beck et al., 1961), Global Assessment Scale (GAS) (Endicott et al., 1976), Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), and Affect Rating Scale (ARS) for affective flattening and affective overreactivity. The administration of the scales coincided with pretreatment and posttreatment platelet receptor binding studies.

All patients received CPZ for 14 days with a dosage range of 200-800 mg/day (average dose = 458 mg/day). All patients who began treatment with CPZ completed the 14-day drug treatment period.

Platelet Isolation and Ligand Binding Assays. Approximately 70 ml of blood were obtained by venipuncture from either schizophrenic patients (mean age = 30.1 ± 12.0 years) or healthy control subjects (mean age = $29.0 \pm \text{SD } 7.6$ years), and the specific binding of ^3H -clonidine and ^3H -yohimbine to platelet membranes was assessed as described previously

(Garcia-Sevilla et al., 1981a). Plasma levels of DA, NE, and E were determined by a single isotope radioenzymatic assay (Peuler and Johnson, 1977). Ligand binding to platelet membranes and plasma catecholamine levels were determined when patients were drug free for at least 2 weeks and after 2 weeks of CPZ treatment. All blood samples were drawn from subjects in a sitting position between 8 and 9 a.m.

Competition Studies. The inhibitory constant (K_i) of CPZ for the α_2 -adrenergic receptor was calculated against the binding of 4.0 nanomolar (nM) ^3H -clonidine and 4.0 nM ^3H -yohimbine on normal human platelets and against 4.0 nM ^3H -clonidine on rat brain hippocampal membranes as described previously (Garcia-Sevilla et al., 1981a).

Analysis of Results. For the statistical evaluations, Student's two-tailed t test was used. The level of significance was chosen as $p = 0.05$. All results are expressed as mean values \pm the standard deviation (SD).

Results

The specific binding of ^3H -clonidine and ^3H -yohimbine to platelet membranes was a saturable process of high affinity. Specific binding represented $89 \pm 3\%$ ($n = 25$) of the total binding for ^3H -clonidine and $92 \pm 2\%$ ($n = 25$) of the total binding for ^3H -yohimbine at concentrations of $4 \times 10^{-9} \text{ M}$ and $2 \times 10^{-9} \text{ M}$, respectively. Scatchard analysis (Scatchard, 1949) of individual saturation curves showed a marked difference in the maximal number of binding sites (B_{max}) for the two radioligands. In one patient, two separate ^3H -clonidine binding sites were observed with different affinities (K_d) and binding capacities.

Schizophrenic patients had lower B_{max} values for the specific binding of ^3H -yohimbine to platelet membranes than did controls (27%, $p < 0.025$) (Table 1). The B_{max} values for ^3H -clonidine were decreased (17%), but not significantly, from control values. The K_d values for the schizophrenic patients were significantly higher than for controls (K_d for ^3H -clonidine, 10.1 ± 2.1 nanomolar (nM), $p < 0.005$; K_d for ^3H -yohimbine, 5.3 ± 0.3 nM, $p < 0.05$).

After 2 weeks of CPZ treatment, the B_{max} values for the specific binding of both ^3H -clonidine and ^3H -yohimbine were decreased significantly from pretreatment values. A 35% decrease in the mean B_{max} for ^3H -clonidine ($p < 0.005$) and a 41% decrease in the mean B_{max} for ^3H -yohimbine ($p < 0.01$) were observed. A significant change in K_d values after CPZ treatment was observed only for ^3H -yohimbine (45%, $p < 0.025$).

Pretreatment plasma E levels were significantly higher than control values (86%, $p < 0.05$), while NE and DA levels were the same as in controls (Table 2). After 2 weeks of CPZ treatment, E levels were significantly decreased compared to pretreatment values ($p < 0.05$) and were within a normal range. Posttreatment levels of both DA and NE were increased from pretreatment levels; however, only the NE increase was statistically significant (105% increase, $p < 0.025$).

The K_i of CPZ was $1.0 \pm 0.3 \times 10^{-7} \text{ M}$ ($n = 4$) for displacement of ^3H -clonidine and $3.7 \pm 1.9 \times 10^{-7} \text{ M}$ ($n = 4$) for displacement of ^3H -yohimbine from the platelet α_2 -adrenergic receptor. The K_i of CPZ, as measured by ^3H -clonidine displacement from rat hippocampal membranes, was similar ($6.4 \pm 2.4 \times 10^{-7} \text{ M}$; $n = 3$).

Table 1. Tritiated clonidine and yohimbine binding to platelet membranes of schizophrenic patients

Patient/Age	Clonidine				Yohimbine			
	Before treatment		After treatment		Before treatment		After treatment	
	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
1/28	30	16.2	12	10.8	53	3.9	48	3.2
2/22	25	10.0	24	33.4	84	3.9	33	7.0
3/36	20	2.3	17	14.4	101	4.4	70	7.6
4/32	24	9.6	17	11.5	94	5.5	38	9.5
5/22	—	—	18	4.4	170	5.7	123	8.6
6/23	23	3.3	19	2.7	151	6.3	114	5.2
7/38	34	21.4	19	48.7	157	4.9	57	14.5
8/27	39	12.5	20	10.0	108	5.8	91	5.2
9/34	39	5.1	19	17.1	161	5.6	61	7.5
10.39	26	10.1	22	24.0	127	6.7	—	—
Mean \pm SD								
(age, 30.1 \pm 12.0 years)	29 \pm 6	10.1 \pm 6.6 ¹	19 \pm 32	17.7 \pm 14.2	121 \pm 38 ³	5.3 \pm 0.94	70 \pm 35 ⁵	7.6 \pm 3.5 ⁶

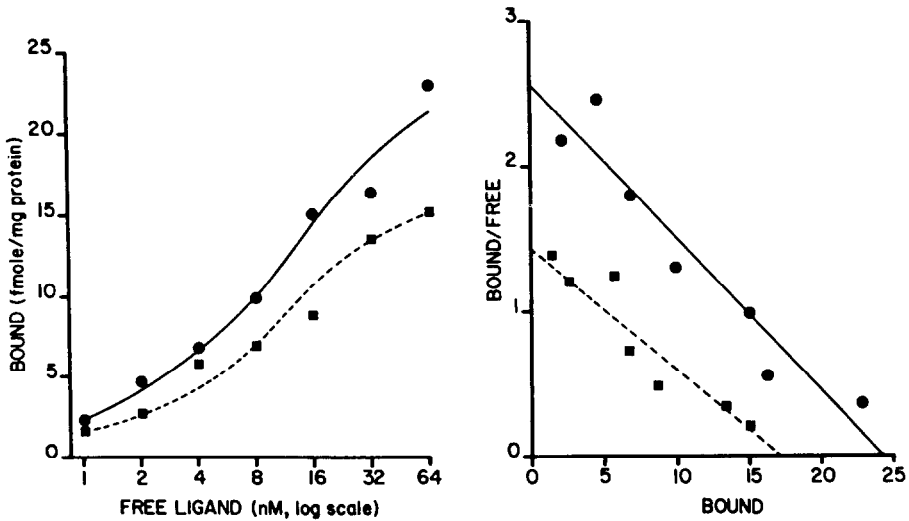
Note. The maximum number of binding sites (B_{max}) and apparent dissociation constants (K_d) were calculated by Scatchard analysis of full saturation curves for platelets from each subject. B_{max} , femtomoles/mg protein; K_d , nanomolar (nM). Control subjects were age-matched (age = 29.0 \pm 7.6 years, n = 16). Control values for ^3H -clonidine binding were: B_{max} = 35 \pm 12 fmole/mg protein; K_d = 5.5 \pm 12.4 nM. Control values for ^3H -yohimbine binding were: B_{max} = 165 \pm 44 fmole/mg protein; K_d = 4.0 \pm 2.0 nM.

1. p < 0.005 when compared with K_d of control subjects.
2. p < 0.005 when compared with pretreatment value.
3. p < 0.025 when compared with B_{max} of control subjects.
4. p < 0.05 when compared with K_d of control subjects.
5. p < 0.01 when compared with pretreatment value.
6. p < 0.025 when compared with pretreatment value.

Discussion

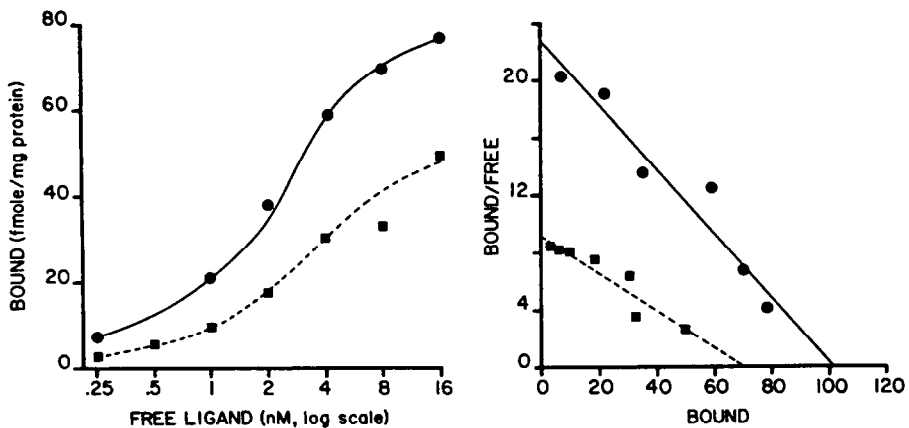
The present study indicates that in chronic schizophrenic patients there is a significant decrease in the number of platelet α_2 -adrenergic receptors when measured by the specific binding of ^3H -yohimbine, an α_2 -adrenergic receptor antagonist. In contrast, the specific binding to platelet membranes from schizophrenic patients of ^3H -clonidine, a partial agonist at α_2 -adrenergic receptors, does not differ from binding of ^3H -clonidine to platelet membranes from control subjects. The specific binding of the two ligands to platelet membranes isolated from patients with other psychiatric disorders differs from that observed in the present study. The specific binding of ^3H -clonidine to platelet membranes has been found to be increased in patients with major depressive disorder (Garcia-Sevilla et al., 1981b). A number of studies have shown that the specific binding of ^3H -yohimbine to platelet membranes is not significantly different in patients with major depressive disorder than in normal subjects (Daiguji et al., 1981; Smith et al., 1983; Stahl et al., 1983). In contrast, patients with panic-anxiety disorder have a decreased number of binding sites on platelet membranes for ^3H -yohimbine but a normal number of binding sites for ^3H -clonidine

Fig. 1. Saturation curves and Scatchard plots for ^3H -clonidine binding to platelet membranes of patient 4 before (circle) and after (square) chlorpromazine treatment



See Table 1 for changes in binding parameters.

Fig. 2. Saturation curves and Scatchard plots for ^3H -yohimbine binding to platelet membranes of patient 3 before (circle) and after (square) chlorpromazine treatment



See Table 1 for changes in binding parameters.

Table 2. Plasma catecholamine levels¹

Patient	Pretreatment			Posttreatment		
	DA	E	NE	DA	E	NE
1	20.5	115.5	473.8	71.6	64.3	702.4
2	0.0	116.7	281.2	67.7	44.3	684.1
3	0.0	201.2	210.0	69.1	47.5	742.9
4	16.1	183.6	559.0	81.0	125.7	939.5
5	24.3	130.6	485.9	40.2	37.8	547.5
6	15.1	22.1	325.0	0.0	0.0	425.1
7	0.0	42.7	489.8	808.1	0.0	1982.7
8	0.0	24.6	464.7	137.7	0.0	1217.2
9	0.0	80.0	281.1	27.1	64.7	355.9
10	27.6	26.7	381.6	9.6	41.8	523.9
Mean	10.4	94.4 ²	395.2	130.2	42.6 ³	812.14
± SD	± 11.3	± 66.1	± 115.7	± 241.3	± 38.6	± 482.6

1. Plasma catecholamine levels are expressed in units of picogram/ml. Control values for catecholamine levels were: dopamine (DA), 12.5 ± 39.6 ; epinephrine (E), 57.0 ± 69.6 ; norepinephrine (NE), 273.8 ± 164.4 .

2. $p < 0.05$ when compared with control subjects.

3. $p < 0.05$ when compared with pretreatment value.

4. $p < 0.025$ when compared with pretreatment value.

(Cameron et al., 1983). There is considerable evidence that adrenergic agonists differ from antagonists in binding to different affinity states of the α_2 -adrenergic receptor on platelet membranes (Garcia-Sevilla et al., 1981a; Bylund and U'Prichard, 1983). Studies such as the present one suggest that the differences between the specific binding of adrenergic agonists and adrenergic antagonists to platelet membranes might be characteristic of certain psychiatric disorders.

The observation in this study of a decreased number of α_2 -adrenergic receptors as measured by ^3H -yohimbine binding differs from the previous report of an increased number of platelet α -adrenergic receptors in schizophrenic patients as measured by the binding of ^3H -dihydroergocriptine, an α -adrenergic receptor antagonist (Kafka and van Kammen, 1983). A number of studies have reported differences in the binding of ^3H -yohimbine and ^3H -dihydroergocriptine to platelet membranes, and Bylund and U'Prichard (1983) have suggested that greater selectivity might exist in the affinity of ^3H -yohimbine binding for the α_2 -adrenergic receptor as compared to ^3H -dihydroergocriptine. There might be other explanations for the differences between the present study and that of Kafka and van Kammen (1983). In that study, the male patients were hospitalized for longer periods of time (average hospitalization = 2.1 ± 0.7 years) than the patients in the present study, who were neither under chronic care nor long-term medications at the time of the study. Since CPZ is a potent α -receptor antagonist, the increase in receptor number reported by Kafka and van Kammen (1983) might be an effect of long-term blockade of α_2 -adrenergic receptors by neuroleptics. Preliminary studies in this laboratory on rats have found an increase in α_2 -receptors in specific brain areas following long-term, but not acute, CPZ treatment (Spengler and Smith, 1982).

Treatment of schizophrenic patients with CPZ for 2 weeks resulted in marked increases in plasma NE content and appreciable decreases in the specific binding of both ^3H -clonidine and ^3H -yohimbine to isolated platelet membranes. Similar increases in plasma NE content in schizophrenic patients treated with CPZ have been reported by other investigators (Naber et al., 1980; Castellani et al., 1982). An increase in plasma NE content has also been observed in both schizophrenic and normal control subjects after treatment with clozapine, a benzodiazepine antipsychotic agent (Sarafoff et al., 1979). These increases in plasma NE content would be expected to result from blockade of the α_2 -adrenergic receptor by CPZ, since this receptor regulates the neuronal release of NE by a feedback inhibitory mechanism (Langer, 1977). Furthermore, CPZ also blocks the neuronal uptake of NE (Barth et al., 1982).

An interesting finding of the present study is the apparent inverse relationship between plasma NE content and the number of α_2 -adrenergic receptors on platelet membranes. Similar relationships between increases in plasma NE levels and decreases in platelet α_2 -receptor number have been observed in patients with congestive heart failure (Weiss et al., 1983) and in patients with panic-anxiety disorder (Cameron et al., 1983) both before and after treatment. Although incubation of platelet membranes with high concentrations of epinephrine for 4 to 22 hours probably does not down-regulate the absolute number of α_2 -adrenergic receptors (Karliner et al., 1982), elevations in circulating NE content over a period of several weeks might cause decreases in receptor number such as those observed in the present study.

The observation that the dissociation constant (K_d) of ^3H -yohimbine increases slightly after CPZ treatment suggests that CPZ might be competitively interacting directly with the α_2 -adrenergic receptor. CPZ displaced ^3H -clonidine and ^3H -yohimbine from platelet membranes with a K_i in the 10^{-7} M range, which is much greater than plasma levels of CPZ as reported in patients undergoing similar drug therapy (Schooler et al., 1976) and suggests that the decrease in receptor number after drug treatment is not due to competition between CPZ and the radioligands. CPZ also displaced ^3H -clonidine from rat hippocampal membranes with a K_i in the 10^{-7} M range, which correlates well with previously reported values for CPZ displacement of ^3H -clonidine in whole rat brain homogenates (Peroutka et al., 1977). These findings indicate that CPZ has a similar affinity for both the neural and platelet receptor.

The results of the present study support earlier findings which suggest that there is abnormal noradrenergic function in schizophrenia (Farley et al., 1978; Hornykiewicz, 1982; Sternberg et al., 1982) and that neuroleptic drugs might act in part through noradrenergic mechanisms. This study also suggests that plasma NE levels might be related to platelet α_2 -adrenergic receptor number, as has been previously suggested for patients with severe congestive heart failure (Weiss et al., 1983) and with panic-anxiety disorder (Cameron et al., 1983). Finally, the study suggests that differences in the binding characteristics of adrenergic agonists and antagonists might be of value in assessing psychiatric disorders and response to drug therapy.

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References

- Barth, N., Manns, M., and Muschol, E. Arrhythmias and inhibition of noradrenaline uptake caused by tricyclic antidepressants and chlorpromazine on the isolated perfused rabbit heart. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **288**, 215 (1975).
- Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., and Erbaugh, J. An inventory for measuring depression. *Archives of General Psychiatry*, **4**, 561 (1961).
- Bunney, B.S. Dopaminergic blocking effects of antipsychotic drugs. *Journal of Psychiatric Research*, **11**, 72 (1974).
- Bylund, D.B., and U'Prichard, D.C. Characterization of α_1 - and α_2 -adrenergic receptors. *International Review of Neurobiology*, **24**, 343 (1983).
- Cameron, O., Hollingsworth, P.J., Nesse, R., Curtis, G., and Smith, C.B. Platelet α_2 adrenoreceptors in panic anxiety (Abstract 55). Presented at the Annual Meeting of the American Psychiatric Association, New York, May (1983).
- Carlsson, A. The impact of catecholamine research on medical science and practice. In: Usdin, E., Kopin, I.J., and Barchas, J., eds. *Catecholamines: Basic and Clinical Frontiers*. Vol. 1. Pergamon Press, Oxford, p. 4 (1979).
- Castellani, S., Ziegler, M.G., van Kammen, D.P., Alexander, P.E., Siris, S.G., and Lake, C.R. Plasma norepinephrine and dopamine- β -hydroxylase activity in schizophrenia. *Archives of General Psychiatry*, **39**, 1145 (1982).
- Crow, T.J., Baker, H.F., Cross, A.J., Joseph, M.H., Lofthouse, R., Longden, A., Owen, F., Riley, G.J., Glover, V., and Killpack, W.S. Monoamine mechanisms in chronic schizophrenia: Post-mortem neurochemical findings. *British Journal of Psychiatry*, **134**, 249 (1979).
- Daiguji, A., Meltzer, H.Y., Tong, C., U'Prichard, D.C., Young, M., and Kravitz, H. α_2 -adrenergic receptors in platelet membranes of depressed patients: No change in number or ^3H -yohimbine affinity. *Life Sciences*, **28**, 2705 (1981).
- Endicott, J., Spitzer, R.L., Fleiss, J.L., and Cohen, J. The Global Assessment Scale. *Archives of General Psychiatry*, **33**, 766 (1976).
- Farley, I.J., Price, K.S., McCullough, E., Deck, J.H., Hordyniski, W., and Hornykiewicz, O. Norepinephrine in chronic paranoid schizophrenia: Above normal levels in limbic forebrain. *Science*, **200**, 456 (1978).
- Garcia-Sevilla, J.A., Hollingsworth, P.J., and Smith, C.B. α_2 -Adrenoreceptors on human platelets: Selective labeling by [^3H] clonidine and [^3H] yohimbine and competitive inhibition by antidepressant drugs. *European Journal of Pharmacology*, **74**, 329 (1981a).
- Garcia-Sevilla, J.A., Zis, A.P., Hollingsworth, P.J., Greden, J.F., and Smith, C.B. Platelet α_2 -adrenergic receptors in major depressive disorder. *Archives of General Psychiatry*, **38**, 1327 (1981b).
- Hamilton, M. A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry*, **23**, 56 (1960).
- Hornykiewicz, O. Brain catecholamines in schizophrenia: A good case for noradrenaline. *Nature*, **299**, 484 (1982).
- Janssen, P.A., and Van Bever, W.F. Preclinical psychopharmacology of neuroleptics. In: Clark, W.G., and del Guidice, J., eds. *Principles of Psychopharmacology*. 2nd ed. Academic Press, Inc., New York (1978).
- Kafka, M.S., and van Kammen, D.P. Alpha-adrenergic receptor function in schizophrenia. *Archives of General Psychiatry*, **40**, 264 (1983).
- Karliner, J.S., Motulsky, H.J., and Insel, P.A. Apparent "down-regulation" of human platelet α_2 -adrenergic receptors is due to retained agonist. *Molecular Pharmacology*, **21**, 36 (1982).
- Langer, S.Z. Presynaptic receptors and their role in the regulation of transmitter release. *British Journal of Pharmacology*, **60**, 481 (1977).
- Naber, D., Finkbeiner, C., Fischer, B., Zander, K.J., and Ackenheil, M. Effect of long-term neuroleptic treatment on prolactin and norepinephrine levels in serum of chronic schizophrenics: Relations to psychopathology and extra-pyramidal symptoms. *Neuropsychobiology*, **6**, 181 (1980).

Overall, J.E., and Gorham, D.R. The Brief Psychiatric Rating Scale. *Psychological Reports*, **10**, 799 (1962).

Peroutka, S.J., U'Prichard, D.C., Greenberg, D.A., and Snyder, S.H. Neuroleptic drug interactions with norepinephrine alpha receptor binding sites in rat brain. *Neuropharmacology*, **16**, 549 (1977).

Peuler, J.D., and Johnson, G.A. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine, and dopamine. *Life Sciences*, **21**, 625 (1977).

Sarafoff, M., Davis, L., and Ruther, E. Clozapine induced increase of human plasma norepinephrine. *Journal of Neural Transmission*, **46**, 175 (1979).

Scatchard, G. The attraction of proteins for small molecules and ions. *Annals of the New York Academy of Sciences*, **51**, 660 (1949).

Schooler, N.R., Sakalis, G., Chan, T.L., Gershon, S., Goldberg, S.C., and Collins, P. Chlorpromazine metabolism and clinical response in acute schizophrenia: A preliminary report. In: Gottschalk, L.A., and Merlis, S., eds. *Pharmacokinetics of Psychoactive Drugs*. New York, Halsted Press, p. 199 (1976).

Smith, C.B., Garcia-Sevilla, J.A., and Hollingsworth, P.J. Alpha₂-adrenoreceptors in rat brain are decreased after long-term tricyclic antidepressant drug treatment. *Brain Research*, **210**, 413 (1981).

Smith, C.B., Hollingsworth, P.J., Garcia-Sevilla, J.A., and Zis, A.P. Platelet alpha₂-adrenoreceptors are decreased in number after antidepressant treatment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **7**, 241 (1983).

Spengler, R.N., and Smith, C.B. Chronic chlorpromazine alters specific binding of tritiated clonidine to membranes from various areas of rat brain. *The Pharmacologist*, **24**, 692 (1982).

Spitzer, R.L., Endicott, J., and Robins, E. Research Diagnostic Criteria: Rationale and reliability. *Archives of General Psychiatry*, **35**, 773 (1978).

Stahl, S.M., Lemoine, P.M., Ciaranello, R.D., and Berger, P.A. Platelet alpha₂-adrenergic receptor sensitivity in major depressive disorder. *Psychiatry Research*, **10**, 157 (1983).

Starke, K. Regulation of noradrenaline release by presynaptic receptor systems. *Review of Physiological and Biochemical Pharmacology*, **77**, 1 (1977).

Starke, K. Presynaptic receptors. *Annual Review of Pharmacology and Toxicology*, **21**, 7 (1981).

Sternberg, D.E., Charney, D.S., Heninger, G.R., Leckman, J.F., Hafstad, K.M., and Landis, D.H. Impaired presynaptic regulation of norepinephrine in schizophrenia. *Archives of General Psychiatry*, **39**, 285 (1982).

Van Kammen, D.P. The dopamine hypothesis of schizophrenia revisited. *Psychoneuroendocrinology*, **4**, 37 (1979).

Weiss, R.J., Tobes, M., Wertz, C.E., and Smith, C.B. Platelet alpha₂ adrenoreceptors in chronic congestive heart failure. *American Journal of Cardiology*, **52**, 101 (1983).