J Neural Transm [GenSect] (1993) 93: 27–35

Journal of Neural Transmission

Concurrence of cortex and platelet serotonin$_2$ receptor binding characteristics in the individual and the putative regulation by serotonin*

S. Ostrowitzki[1], **M. L. Rao**[1], **J. Rédei**[2], and **A. H. Andres**[1]

[1] Psychiatrische Klinik, and [2] Institut für Medizinische Statistik, Dokumentation und Datenverarbeitung, Rheinische Friedrich-Wilhelms-Universität, Bonn, Federal Republic of Germany

Accepted February 3, 1993

Summary. Changes in the central and peripheral serotonergic receptor activity have been reported to be involved in depression and suicidality. To elucidate the interdependence between central and peripheral receptor sites and their regulation by serotonin, we estimated intra-individual serotonin$_2$ receptor binding characteristics in porcine cortex synaptosomes and in platelet membranes using ^{3}H-LSD as ligand and ketanserin as competitor and quantified the relevant serotonin concentrations. A positive correlation between the apparent half maximal saturation concentration, K_D, of the receptor in cortex synaptosomes and platelet membranes ($r = 0.65$, $p = 0.0046$, $n = 18$), and between the apparent maximal binding capacity, B_{max}, of the receptor in cortex synaptosomes and platelets ($r = 0.52$, $p = 0.027$, $n = 18$) was observed. The blood serotonin concentrations correlated negatively with the maximal binding capacity, B_{max}, in platelets ($r = -0.77$, $p = 0.0002$, $n = 18$). These results suggest that the binding characteristics of the central and peripheral serotonin$_2$ receptor are similar, and that the platelet receptor activity may be regulated by blood levels of serotonin.

Keywords: Serotonin$_2$ receptor binding characteristics, serotonin concentrations, brain cortex synaptosomes, platelet membranes, intra-individual correlation, pig.

Introduction

The serotonin$_2$ receptor has been pharmacologically characterized in the mammalian frontal cortex and blood platelets. The turnover of serotonin by platelets

---

* This paper is part of the Inaugural Dissertation of S. Ostrowitzki, Medizinische Fakultät, University Bonn. The results have been presented at the AGNP meeting 1991 in Nürnberg, Germany. The study is supported in part by a grant from the Braun-Stiftung

is considered to serve as peripheral model of central serotonergic neurons (Sneddon, 1973; Stahl and Meltzer, 1978). Alterations of the activity of the serotonin$_2$ receptor in both tissues have been implicated in depression and aggressive behaviour (Arora and Meltzer, 1989; Aprison et al., 1978; Mann et al., 1986; McKeith et al., 1987; Pandey et al., 1990; Stanley and Mann, 1983; Yates et al., 1990). However, little is known with respect to in vivo finding concerning the regulatory influence of the endogenous concentrations of the ligand serotonin on serotonin receptors.

Despite the postulate that platelets serve as a peripheral model for brain serotonergic neuron function intra-individual studies using an identical protocol to compare serotonin$_2$ receptor binding characteristics in both tissues have not been carried out. To examine these issues we studied intra-individual brain and platelet serotonin$_2$ receptor binding characteristics and related these to their respective blood serotonin concentrations.

Materials and methods

The present study was carried out with brain specimen and blood samples obtained from 18 German female pigs from the local abattoir immediately after sacrifice. Wherever applicable, the procedures were carried out using an identical protocol.

Brain cortex synaptosomes

Porcine (Deutsche Landrasse) brain (n = 18) was transported on ice from the local abattoir to the laboratory. The frontal cortex (3 – 5 g) was dissected ventrally of the chiasma opticum on the right hemisphere. All procedures were carried out at 4 °C. The synaptosomes were prepared according to Creese and Snyder (1977). The brain cortical tissue was cut into small pieces, suspended and homogenized in 80 ml of buffer I (50 mmol/L Tris-HCl, pH 7.7). The homogenate was sonicated in ice-water for 45 s with a Braun sonicator (Sonic 300 S, Braun, Melsungen, Germany) and filtered through 45 µ nylon gauze. The filtrate was centrifuged for 20 min at 20 000 **g** (MSE Superspeed 75, Measuring Scientific Equipment LTD, London, England). The sediment was suspended in buffer I, homogenized and centrifuged twice as described above. The final pellet was resuspended and homogenized in buffer II (50 mmol/L Tris-HCl, pH 7.4, containing 120 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$ and 0.05% ascorbic acid), resulting in 200 mg wet weight/ml. The synaptosomes were stored at – 80 °C until assayed for receptor studies.

Platelet membranes

We collected also from each animal 100 ml blood into siliconized glass tubes containing 5 ml 5.5 mmol/L EDTA. All subsequent procedures were carried out at 4 °C. The blood was centrifuged after 45 min for 15 min at 200 **g** (Minifuge 2, Heraeus Christ, Osterode, Germany) to obtain platelet-rich plasma. The supernatant was centrifuged for 10 min at 4000 **g** to prepare platelet-poor plasma and platelets. Platelet membranes were prepared by the method of Pandey et al. (1990). In brief, the platelets were suspended in buffer III (5 mmol/L Tris-HCl, containing 0.1% EDTA, pH 7.5) and centrifuged for 10 min at 4000 **g**. The sediment was resuspended in 4 ml of buffer III and stored at – 80 °C until used for binding studies. Prior to the assay the platelet membranes were thawed on ice, homogenized in a loose-fitting Potter Elvehjem glass homogenizer (Braun, Melsungen, Germany) and centrifuged for 20 min at 30 000 **g**. The membrane pellet was suspended in buffer III,

homogenized as described above and centrifuged for 20 min at 30 000 **g**. The final membrane pellet was suspended in 3.5 ml of buffer II, homogenized and sonicated for 25 s in ice-water.

The final protein concentration of the synaptosomal and platelet membrane preparations was adjusted to 1 mg protein/ml.

Platelets were counted in a Neubauer chamber; the results were validated by external quality control.

Binding assay

The serotonin$_2$ receptor binding assays were carried out in triplicate in siliconized glass tubes, with 150 µl buffer II and 100 µl membrane suspension, corresponding tubes to 100 µg protein. The reaction was started with the addition of 50 µl ^{3}H-LSD in 6 different final concentrations ranging from 0.08 to 5.0 nmol/L corresponding to 1700–100 000 cpm. The final volume of the incubation was 300 µl. Nonspecific binding was determined by replacing 50 µl buffer II with a non-radioactive competitor, the serotonin$_2$ receptor antagonist, ketanserin in buffer II in a final concentration of 1 µmol/L. Specific binding was the difference between the binding in the absence and presence of ketanserin. Incubations were performed for 3 h at 37 °C. The incubation was terminated by rapid filtration through Whatman GF/C filters; the filters were washed twice with ice-cold buffer II and counted in 5 ml Aquasafe 300 (Zinsser Analytic, Frankfurt, Germany) in a liquid scintillation beta-counter (Packard, Frankfurt, Germany).

Protein determination

Protein was determined with a modified method of Lowry et al. (1951) using bovine serum albumin as standard.

Quantification of serotonin

Serotonin was measured by high pressure liquid chromatography using electrochemical detection, in blood, platelet rich plasma, and frontal cortex homogenate (Rao and Fels, 1987). The intra- and interassay coefficients of variation were 2.9 and 4.4%, respectively. The lower limit of detection was 5 nmol/L.

Statistics

All experiments were carried out in triplicate. The average coefficient of variation over the entire concentration range of the binding experiment was 4.9 ± 2.6% (mean ± SD). Binding parameters were analyzed according to Scatchard (1949) using linear regression analysis; the average correlation coefficient r was -0.95 ± 0.05 and -0.95 ± 0.04, ($n = 18$) for the binding experiments with synaptosomes and platelet membranes, respectively. Linear regression analysis (Pearson's linear correlation coefficient) was performed to assure correlations.

Results

Serotonin$_2$ receptor binding in porcine frontal cortex synaptosomes and platelet membranes was saturable and possessed high affinity (Table 1). From the time and temperature curve we inferred that the specific binding was highest at 37 °C, the equilibrium was attained after 3 h at 37 °C. The Scatchard plots of the specific binding were linear, indicating the presence of a single binding site in both tissue preparations in the concentration range between 0.08 to 5 nmol/L ^{3}H-LSD (Fig. 1 A and B).

Table 1. Binding characteristics of the serotonin$_2$ receptor and serotonin concentrations (n = 18)

Parameter	Mean ± SD	Range
Brain cortex		
Serotonin$_2$ receptor, synaptosomes		
B_{max} (fmol/mg protein)	209.1 ± 60.6	122.7 − 322.5
K_D (nmol/L)	0.99 ± 0.64	0.44 − 2.54
Serotonin, homogenate (nmol/g wet weight)	0.832 ± 0.53	0.344 − 1.86
Blood		
Serotonin$_2$ receptor, platelet membranes		
B_{max} (fmol/mg protein)	77.8 ± 32.9	43.6 − 159.4
K_D (nmol/L)	0.56 ± 0.37	0.29 − 1.7
Serotonin, blood (μmol/L)	3.65 ± 1.26	1.25 − 6.05
Serotonin (nmol/10^9 platelets)	14.35 ± 3.90	7.85 − 19.95
Platelet count (μl^{-1})	253000 ± 54088	119000 − 331000

At a concentration of 0.2 nmol/L ^{3}H-LSD the specific binding of the receptor in brain cortex synaptosomes was 67 ± 9% (n = 18) of the total binding and 76 ± 12% (n = 18) using platelet membranes. At the lowest concentration used, i.e., at 0.08 nmol/L ^{3}H-LSD, the total binding amounted to 9.8 ± 3.9% and 6.3 ± 2.7% of the radioactivity incubated with brain cortex synaptosomes and platelet membranes, respectively.

The apparent maximal binding capacity, B_{max}, and the apparent half maximal saturation concentration, K_D, of the receptor in brain cortex synaptosomes and platelet membranes displayed a bimodal distribution in that both parameter estimates were higher in the brain than in platelet membranes. There was no interdependence between the apparent maximal binding capacity and the half maximal saturation concentration of the serotonin$_2$ receptor in either tissue.

The maximal binding capacity, B_{max}, of the serotonin$_2$ receptor in brain cortex synaptosomes and in platelet membranes correlated ($r = 0.52$, $p = 0.027$, $n = 18$); this applied also to the receptor's half maximal saturation concentration, K_D, in both tissues ($r = 0.65$, $p = 0.0046$, $n = 18$). Blood and cortex homogenate serotonin concentrations showed an inhomogeneous correlation ($r = -0.49$, $p = 0.04$, $n = 18$). The cortex homogenate serotonin concentrations did not correlate with the synaptosomal receptor's maximal binding capacity. However, we noted a linear correlation between blood serotonin levels and the

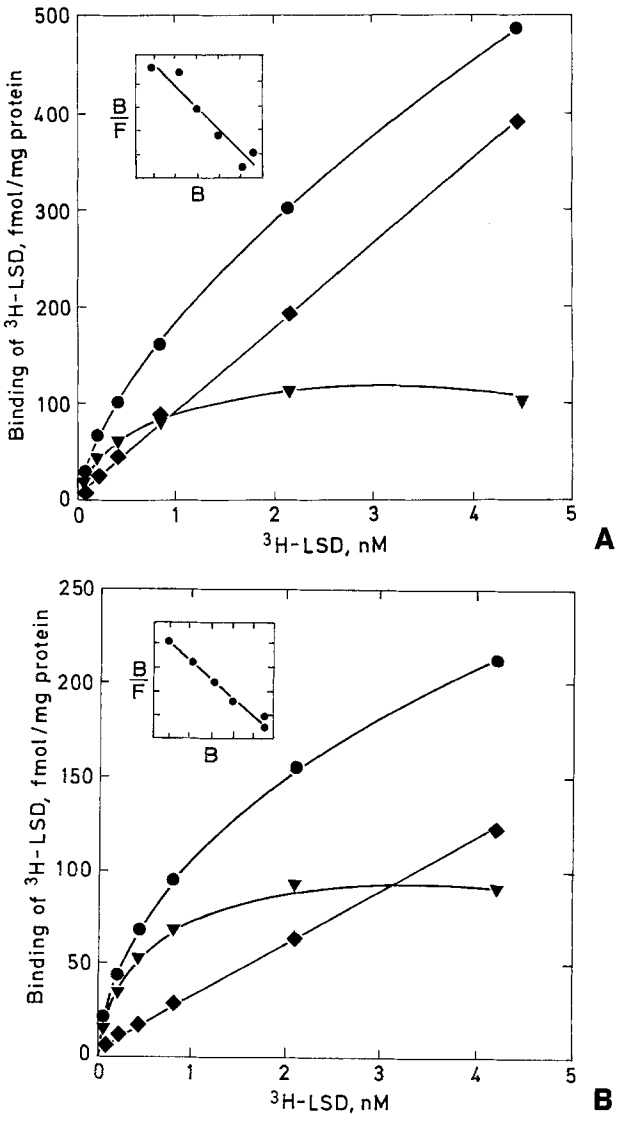

Fig. 1. Binding of ^{3}H-LSD to serotonin$_2$ receptors in **A** porcine frontal cortex synaptosomes and **B** porcine platelet membranes. ● Total binding; ◆ unspecific binding; ▼specific binding

maximal binding capacity of the receptor of platelet membranes ($r = -0.77$, $p = 0.0002$, $n = 18$, Fig. 2).

Discussion

The mammalian frontal cortex comprises the highest serotonin$_2$ receptor density in the brain (Leysen et al., 1981; Engel et al., 1984; Hoyer et al., 1986; Cheetham et al., 1988). Serotonin$_2$ receptors on serotonergic neurons and platelets are coupled to the phosphoinositol pathway (Conn and Sanders-Bush, 1986; Mikuni

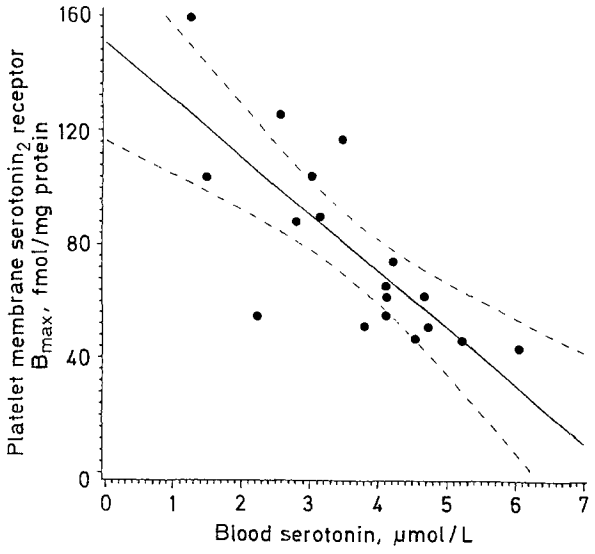

Fig. 2. Correlation of the apparent maximal binding capacity, B_{max}, of the serotonin$_2$ receptor in platelet membranes with blood serotonin concentrations. Dashed lines indicate the 95% confidence interval

et al., 1991). It has been shown that ^{125}I-LSD binds to human cortex ($K_D = 0.35 \pm 0.02$ nM) and platelets ($K_D = 0.37 \pm 0.03$ nM) with similar affinity. Moreover, the inhibition profiles correlated between these compartments (Elliott and Kent, 1989), but these studies were not carried out within an intra-individual set-up.

We chose the pig as animal model since porcine and human brain display similar pharmacological profiles of the serotonin$_2$ receptors (Hoyer et al., 1986). Since it is controversial whether lateralisation of the receptor exists (Yates et al., 1991; Arató et al., 1991), we decided to use the right hemisphere. On account of the size of porcine brain, the cortex synaptosomes of an individual can be prepared in sufficient quantity to estimate the binding characteristics of the serotonin$_2$ receptor. Moreover, from the same animal adequate amounts of blood can be obtained to prepare platelet membranes; this permits the intra-individual comparison of the serotonin$_2$ receptor's activity from frontal cortex and platelet membranes.

The salient findings of this study on central and peripheral serotonin$_2$ receptors are the positive correlations observed between the apparent half maximal saturation concentration, K_D, of the serotonin$_2$ receptor in cortex synaptosomes and platelet membranes, and the apparent maximal binding capacity, B_{max}, of brain cortex synaptosomes and platelet membranes. In addition we noted that blood serotonin concentrations correlate negatively with the B_{max}-values of the serotonin$_2$ receptor of the platelet membranes. This suggests that serotonin is a regulatory factor in that it causes a down-regulation of the serotonin$_2$ receptor at high endogenous concentrations in the periphery. A similar interdependence

between the serotonin concentration and the change in maximal binding capacity of the serotonin$_2$ receptor was not found in the frontal cortex. This could be due to the inhomogeneous distribution of serotonin in brain tissue, since for technical reasons the brain tissue used for the determination of serotonin and for the isolation of the synaptosomes was not identical.

On the other hand, the regulation of the serotonin$_2$ receptor in mammalian brain seems to be unique, in that denervation or chronic blockade do not provoke receptor supersensitivity or up-regulation in mammalian brain (Leysen et al., 1983, 1986; Conn and Sanders-Bush, 1987). Moreover, a down-regulation of the serotonin$_2$ receptor is perceptible after antagonist treatment, i.e., with ketanserin (Leysen und Pauwels, 1990) as well as after agonist (methoxyphenethylamine) treatment (McKenna et al., 1989). Taken together these results do not appear to be consistent with the generally accepted hypothesis on receptor regulation according to which for example β-adrenergic and cholinergic muscarinergic receptors are supersensitive following stimulus deprivation and are desensitized after agonist treatment (Leysen, 1990). Leysen (1990) speculated that under physiological conditions the serotonin$_2$ receptor might receive little stimulation and hence exists in a state of supersensitivity that permits solely a one-way down-regulation in emergencies.

Serotonin$_2$ receptor regulation may proceed disparately during distinct stages of receptor synthesis and receptor life. There is evidence, that alterations in the receptor level are due to changes on the transcriptional level, i.e., increased mRNA levels (Roth et al., 1990 a). On the other hand, following mianserin-treatment, a dissociation between receptor and mRNA levels was noted which points to post-transcriptional regulation. In addition post-translational regulatory mechanisms may occur such as receptor internalization and degradation, as well as modifications on account of receptor phosphorylation, glycosylation or proteolysis (Roth et al., 1990 b). Overall changes in membrane fluidity may also be responsible for alterations in the maximal binding capacity (Heron et al., 1980). In our study, increasing blood serotonin concentrations seem to down-regulate the maximal binding capacity, B_{max}, of the serotonin$_2$ receptor on platelets. The underlying mechanism is not clear; the receptor might be internalized when endogenous serotonin levels are high, but any of the above mentioned mechanisms could be of importance.

We interprete our findings of similar receptor binding characteristics in brain and on peripheral blood cells in that the expression of the serotonin$_2$ receptor might be determined by similar – yet unknown – transcriptional, post-transcriptional or post-translational events in the brain cortex and in platelets. The fact that the maximal binding capacity and the half maximal saturation concentration of the serotonin$_2$ receptor in frontal cortex and in platelets show bimodal distribution might indicate that there are also independent regulatory mechanisms. The intra-individual correlation between the B_{max} of the synaptosomal and corresponding platelet receptors and between the K_Ds supports the notion that platelets serve as an experimental model to assess the function

of central serotonergic neurons. Thus, findings of alterations of the platelet serotonin$_2$ receptors in psychiatric diseases might provide information on the function of frontal cortex serotonin$_2$ receptor.

Acknowledgements

The authors are grateful for the expertise of C. Frahnert, M. Krämer and G. Netthövel in the initial stage of the investigation and to C. Frahnert and G. Netthövel for the determination of serotonin. The gift of ketanserin by Janssen Pharmaceuticals is gratefully acknowledged.

References

Aprison MH, Takahashi R, Tachiki K (1978) Hypersensitive serotonergic receptors in clinical depression – a theory. In: Haber B, Aprison MH (eds) Neuropharmacology and behaviour. Plenum, New York, pp 23–53

Arató M, Frecska E, Tekes K, Maccrimmon D (1991) Serotonergic interhemispheric asymmetry: gender difference in the orbital cortex. Acta Psychiatr Scand 84 (1): 110–111

Arora RC, Meltzer HY (1989) Increased serotonin$_2$ (5-HT$_2$) receptor binding as measured by ^{3}H-lysergic acid diethylamide (^{3}H-LSD) in the blood platelets of depressed patients. Life Sci 44: 725–734

Cheetham SC, Crompton MR, Katona CLE, Horton RW (1988) Brain 5-HT$_2$ receptor binding-sites in depressed suicide victims. Brain Res 443: 272–280

Conn PJ, Sanders-Bush E (1986) Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT-2 binding site. J Neurosci 6: 3669–3675

Conn PJ, Sanders-Bush E (1987) Central serotonin receptors: effector systems, physiological roles and regulation. Psychopharmacology 92: 267–277

Creese I, Snyder SH (1977) A simple and sensitive radioreceptor assay for antischizophrenic drugs in blood. Nature 270: 180–182

Elliott JM, Kent A (1989) Comparison of [^{125}I]iodolysergic acid diethylamide binding in human frontal cortex and platelet tissue. J Neurochem 53 (1): 191–196

Engel G, Müller-Schweinitzer E, Palacios JM (1984) 2-[^{125}I]LSD, a new ligand for the characterisation and localisation of 5-HT$_2$ receptors. Naunyn Schmiedebergs Arch Pharmacol 325: 328–336

Heron DS, Shinitzky M, Hershkowitz M, Samuel D (1980) Lipid fluidity markedly modulates the binding of serotonin to mouse brain membranes. Proc Natl Acad Sci 77: 7463–7467

Hoyer D, Pazos A, Probst A, Palacios JM (1986) Serotonin receptors in the human brain. II. Characterisation and autoradiographic localisation of 5-HT$_{1C}$ and 5-HT$_2$ recognition sites. Brain Res 376: 97–107

Leysen JE (1990) Gaps and peculiarities in 5-HT$_2$ receptor studies. Neuropsychopharmacology 3: 361–369

Leysen JE, Pauwels PJ (1990) 5-HT$_2$ receptor, roles and regulation. Ann NY Acad Sci 600: 183–193

Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J, Janssen PAJ (1981) Receptor binding profile of R 41468, a novel antagonist at 5-HT$_2$ receptors. Life Sci 28: 1015–1022

Leysen JE, Van Gompel P, Verwimp M, Niemeggers CJE (1983) Role and localization of serotonin (S_2)-receptor-binding sites: effects of neuronal lesions. In: Mandel P, DeFeudis FV (eds) CNS receptors – from molecular pharmacology to behaviour. Raven, New York, pp 373–383

Leysen JE, Van Gompel P, Gommeren W, Woestenborghs R, Janssen PAJ (1986) Down regulation of serotonin-S_2 receptor sites in rat brain by chronic treatment with the serotonin-S_2 antagonists: ritanserin and setoperone. Psychopharmacology 88: 434–444

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275

Mann JJ, Stanley M, McBride A, McEwen BS (1986) Increased serotonin$_2$ and β-adrenergic receptor binding in the frontal cortices of suicide victims. Arch Gen Psychiatry 43: 954–959

McKeith IG, Marshall EF, Ferrier IN, Armstrong MM, Kennedy WN, Perry RH, Perry EK, Eccleston J (1987) 5-HT receptor binding in post-mortem brain from patients with affective disorder. J Affect Disord 13: 67–74

McKenna DJ, Nazarali AJ, Himeno A, Saavedra JM (1989) Chronic treatment with (±) DOI, a psychomimetic 5-HT_2 agonist, downregulates 5-HT_2 receptors in rat brain. Neuropsychopharmacology 2: 81–87

Mikuni M, Kusumi I, Kagaya A, Kuroda Y, Mori H, Takahashi K (1991) Increased 5-HT-2 receptor function as measured by serotonin-stimulated phosphoinositide hydrolysis in platelets of depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 15: 49–61

Pandey GN, Pandey SC, Janicak PG, Marks RC, Davis JM (1990) Platelet serotonin-2 receptor binding sites in depression and suicide. Biol Psychiatry 28: 215–222

Rao ML, Fels K (1987) Beeinflussen Tryptophan und Serotonin beim Menschen die Melatonin-Ausschüttung des „Regulators der Regulatoren" (Zirbeldrüse)? Fortschr Pharmakother 3: 87–99

Roth BL, Hamblin MW, Ciaranello RD (1990 a) Developmental regulation of 5-HT_2 and 5-HT_{1C} mRNA and receptor levels. Dev Brain Res 58: 51–58

Roth BL, Hamblin MW, Ciaranello RD (1990 b) Regulation of 5-HT_2 and 5-HT_{1C} serotonin receptor levels: methodology and mechanisms. Neuropsychopharmacology 3: 427–433

Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51: 660–672

Sneddon JM (1973) Blood platelets as model for monoamine containing neurons. Prog Neurobiol 1: 151–197

Stahl SM, Meltzer HY (1978) A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: comparison with central serotonergic neurons. J Pharmacol Exp Ther 205: 118–132

Stanley M, Mann JJ (1983) Increased serotonin-2 binding sites in frontal cortex of suicide victims. Lancet i: 214–216

Yates M, Leake A, Candy JM, Fairbairn AF, McKeith IG, Ferrier IN (1990) 5HT_2 receptor changes in major depression. Biol Psychiatry 27: 489–496

Yates M, Morris C, Cheng AV, Ferrier IN (1991) Laterality and 5HT_2 receptors in human brain. Psychiatry Res 36: 169–174

Authors' address: Prof. M. L. Rao, Psychiatrische Klinik, Rheinische Friedrich-Wilhelms-Universität, Sigmund-Freud-Strasse 25, D-53127 Bonn, Federal Republic of Germany.

Received August 11, 1992