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# Serotonin 5-$HT_{2A}$ Receptor Binding in Platelets from Healthy Subjects as Studied by [$^3$H]-Lysergic Acid Diethylamide ([$^3$H]-LSD): Intra- and Interindividual Variability

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*In studies on platelet 5-$HT_{2A}$ receptor binding in patients with neuropsychiatric disorders, there has been a marked variability and a considerable overlap of values between patients and controls. The causes of the large variability in 5-$HT_{2A}$ receptor parameters is still unsettled. In the present study, we have quantified the intra- and interindividual variability of platelet 5-$HT_{2A}$ receptor binding in 112 healthy subjects and explored factors that may influence 5-$HT_{2A}$ receptor binding, using [$^3$H]-lysergic acid diethylamide as radioligand. Age, gender, blood pressure, and metabolic capacity of the liver enzymes CYP2D6 and CYP2C19 did not influence $B_{max}$ and $K_d$ values. Body weight and body mass index (BMI) showed a negative correlation with $K_d$ (p = .04 and .03, respectively), but not with $B_{max}$. $B_{max}$ was significantly lower in the light half of the year than in the dark half of the year (p = .001), and $K_d$ was significantly lower in the fall than in the summer and winter (p < .001). In females, there was a significant increase in $B_{max}$ from week 1 to week 2 of the menstrual cycle (p = .03). Females taking contraceptive pills had significantly higher $K_d$ than drug-free females in weeks 1 and 4 of the menstrual cycle (p = .04). This study shows that a number of factors should be taken into account when using platelet 5-$HT_{2A}$ receptor binding in studies of neuropsychiatric disorders.* ***[Neuropsychopharmacology 16:285–293, 1997]*** *© 1997 American College of Neuropsychopharmacology*

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Alterations in the function of the neurotransmitter serotonin and its receptors are believed to play an important role in the pathogenesis of several neuropsychiatric disorders. Hitherto, seven major classes of serotonin receptors (5-$HT_1$ to 5-$HT_7$) and their subtypes have been described (Peroutka 1995). They are defined by their relative affinities for serotonin agonists and antagonists as well as by their second-messenger systems and by cloning of the receptors.

In human blood platelets, a 5-$HT_{2A}$ receptor with drug affinities and other characteristics similar to those of the 5-$HT_{2A}$ receptor in human frontal cortex has been characterized (McBride et al. 1983; Geaney et al. 1984; Da Prada et al. 1988; Elliott and Kent 1989). Platelet and frontal cortex 5-$HT_{2A}$ receptors are both linked to the phosphoinositide second-messenger system (De Courcelles et al. 1985; Conn and Sanders-Bush 1986), and

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their nucleotide sequences are identical, except for nucleotide 102 (Cook et al. 1994). Moreover, a positive correlation between interindividual 5-$HT_{2A}$ receptor binding characteristics in brain cortex and in platelets has been observed in both animals (Ostrowski et al. 1993) and humans (Andres et al. 1993).

Mounting evidence has suggested that changes in platelet 5-$HT_{2A}$ receptor characteristics exist in a number of neuropsychiatric disorders, including depression (Biegon et al. 1987; Arora and Meltzer 1989a; Pandey et al. 1990), schizophrenia (Arora and Meltzer 1993; Pandey et al. 1993), autistic disorder (McBride et al. 1989), panic disorder (Norman et al. 1990), suicidal behavior independent of diagnosis (Pandey et al. 1995), and migraine (Ribiero et al. 1990; Govitrapong et al. 1992). However, a study in patients with major depressive disorder could not reveal any significant changes in 5-$HT_{2A}$ receptor binding characteristics (McBride et al. 1994). In patients with major depression as well as in suicidal patients, there also is evidence for an increase in the density of 5-$HT_{2A}$ receptors in frontal cortex (McKeith et al. 1987; Arora and Meltzer 1989b; Arango et al. 1990; Yates et al. 1990). Because of the similarities in platelet and brain 5-$HT_{2A}$ receptor characteristics, and because platelets are readily accessible, the platelet 5-$HT_{2A}$ receptor is an attractive model for the study of central 5-$HT_{2A}$ receptor function in neuropsychiatric disorders.

Several radioligands have been used to study the platelet 5-$HT_{2A}$ receptor. Most investigators have used [$^3$H]-lysergic acid diethylamide ([$^3$H]-LSD) (Geaney et al. 1984; Cowen et al. 1986; Arora and Meltzer 1989a; Arora and Meltzer 1993; Cook et al. 1993) or [$^{125}$I]-iodo-LSD (McBride et al. 1987, 1989, 1994; Pandey et al. 1990, 1993, 1995; Sheline et al. 1995), but [$^3$H]-ketanserin (Biegon et al. 1987), and occasionally [$^3$H]-spiperone (Ribiero et al. 1990; Govitrapong et al. 1992) or [$^{125}$I]-iodospiperidol (Perry et al. 1991) have also been used. Subsequently, LSD has been shown to be a more appropriate 5-$HT_{2A}$ receptor ligand than ketanserin (Steckler et al. 1993).

In all studies that have reported abnormal 5-$HT_{2A}$ receptor characteristics in patients with neuropsychiatric disorders, there have been a marked variability and a considerable overlap of values between patients and controls. This variability may be attributed to factors such as age, gender, body weight, blood pressure, hormonal status, and diurnal and seasonal variations. Studies on a limited number of volunteers (usually 20–40 subjects) or patients have, at least in part, given conflicting results, and the causes of the large variability on 5-$HT_{2A}$ receptor characteristics is still unsettled. In the present study, we quantify the intra- and interindividual variability of platelet 5-$HT_{2A}$ receptors and explore a number of factors that may influence 5-$HT_{2A}$ receptor characteristics in a large number of healthy subjects.

## MATERIAL AND METHODS

### Subjects

A total of 112 healthy volunteers, 39 males and 73 females, took part in the investigation, which was approved of by the Ethics Committee of the University of Umeå. Most subjects were medical students or hospital employees, although some were recruited from the community. The mean age of the volunteers was 38.9 years, with a range from 18 to 79 years. Among the females, 22 were postmenopausal and 51 were of childbearing age. The study lasted for 9 months (from June to February), and most samples were collected during the fall.

All subjects were healthy, as assessed by medical history, a physical examination including blood pressure in the supine position, and routine blood chemistry tests. All subjects denied a history of psychiatric disorder, and they had not taken any medications for at least 2 weeks prior to the study. There is some evidence that serotonergic transmission and the capacity of some liver enzymes might be functionally linked (Martinez et al. 1995), and that there might be a relationship between personality and liver enzyme capacity (LLerena et al. 1993). We therefore tested all subjects with regard to the metabolic capacity of the liver enzymes CYP2D6 and CYP2C19 by means of dextromethorphan and mephenytoin, respectively (Spigset et al. in press).

Five males and six females (21–27 years old) took part in a follow-up study in which blood samples were obtained once a week during a 4-week period. All females had regular periods with a duration of 26 to 30 days. In addition, six healthy women (21–24 years old) using contraceptive pills participated in this part of the study. Four of them were taking a combination of etinylestradiol and desogestrel, one was taking etinylestradiol and norethisterone, and one etinylestradiol and levonorgestrel.

### Membrane Preparation

Blood from the antecubital vein was obtained using a 20-gauge needle and collected into 5 polyethylene tubes (Monovette, Sarstedt, Nümbrecht, Germany), containing 1.6 mg/ml blood EDTA (ethylenediamidetetraacetate). Total blood volume collected was 37.5 ml. Single samples were drawn between 8:00 A.M. and 4:00 P.M. In the 4-week follow-up study all samples were obtained between 8:00 A.M. and 9:00 A.M.

Preparation of membranes and assay of [$^3$H]-LSD binding was performed using the method of Geaney et al. (1984) with some modifications. In short, platelet-rich plasma was obtained by centrifugation at 180 × g for 15 minutes at 20°C. The platelet-rich plasma was then centrifuged at 1,200 × g for 10 minutes at 10°C.

The platelet pellet was frozen and kept at −80°C for at least 24 hours (but less than 4 months) until use. At the day of analysis, the platelet pellet was resuspended in hypotonic Tris-buffer (5 mM Tris-HCl, 0.1% EDTA, pH 7.5, 4°C). This suspension was gently homogenized by 20 strokes of a hand-driven glass/glass homogenizer at 4°C and was centrifuged at 30,000 × g for 15 minutes at 4°C. The resulting membrane pellet was washed once more in hypotonic Tris-buffer, homogenized, and centrifuged as above, and then resuspended in the incubation buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, pH 7.5, 4°C).

### [$^3$H]-LSD Binding Assay

[$^3$H]-LSD was purchased from DuPont N.E.N. (Boston, MA, USA). Specific activity of the two lots used was 78.2 and 76.7 Ci/mmol, respectively. 200 µl of the membrane suspension, 25 µl [$^3$H]-LSD, and 25 µl incubation buffer were incubated in microtiter plates for 4 hours at 37°C. Seven concentrations of [$^3$H]-LSD ranging from 0.25 to 2.5 nM were used (Figure 1). In a separate experiment, ketanserin, mianserin, and spiperone in concentrations from 0.1 pM to 100 µM were investigated as displacers. Of these, spiperone was the most appropriate, and a concentration of 300 nM was sufficient for total displacement. Therefore, in the present study, spiperone (Sigma, St. Louis, MO, USA), dissolved in the incubation buffer at a final concentration of 300 nM, was used as displacer. Specific binding was defined as total binding minus binding in the presence of spiperone (Figure 1). All assays were performed in triplicate. The binding was terminated by filtration through Whatman GF/F filters (Whatman, Maidstone, Kent, UK) using a Micro Cell Harvester (Skatron, Labasco, Partille, Sweden). The filters were washed for 30 seconds with 50 nM Tris-buffer and dried. Radioactivity was counted in 4 ml Ready Protein Plus scintillation fluid (Beckman, Fullerton, CA, USA), using a Beckman LS 1801 scintillation counter (Beckman, Fullerton, CA, USA). Total bound [$^3$H]-LSD did not exceed 2% of the total radioactivity. The protein content was assayed by the method of Lowry et al. (1951), modified according to Markwell et al. (1978), using a Ultraspec 4050 spectrophotometer (LKB Biochrome, Cambridge, UK).

### Data Analysis and Statistics

The binding characteristics of [$^3$H]-LSD were calculated from Scatchard analysis of the specific binding data, using means of triplicates, according to the method of least-squares linear regression (Figure 1). For comparative statistics, paired or unpaired Student's *t*-tests, Pearson regression analysis, and analysis of variance (ANOVA) were used. As $K_d$ was found to be log-normally distributed (see later), we have used the log-transformed values of $K_d$ in regression analyses and statistical evaluations. *p* Values lower than .05 were regarded as statistically significant.

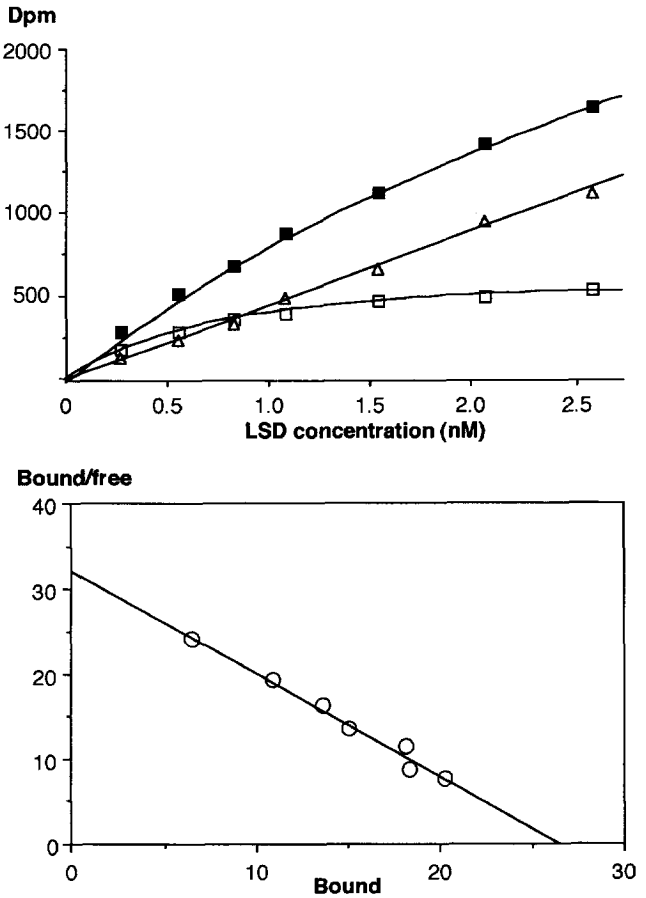


**Figure 1.** *Upper panel;* binding of [$^3$H]-LSD to platelet membranes in a representative experiment (Dpm = disintegrations per minute). *Solid squares,* total binding; *open squares,* specific binding; *triangles,* binding in the presence of spiperone. *Lower panel;* Scatchard analysis of the same experiment (Bound = Specific binding of LSD to platelet membranes, fmol/mg protein; free = unbound LSD concentration, nM). In this experiment, $B_{max}$ = 26.6 fmol/mg protein; $K_d$ = 0.83 nM. $y = 32.2–1.21x$; $r = 0.99$.

## RESULTS

The reliability of the determination of $B_{max}$ and $K_d$ was studied in split duplicates. The mean intraday coefficient of variation was 6.4% for $B_{max}$ and 9.2% for $K_d$. The mean interday coefficient of variation was 7.7% for $B_{max}$ and 11.7% for $K_d$. Storage at −80°C for 4 months did not affect $B_{max}$ or $K_d$ values.

**Table 1.** $B_{max}$ and $K_d$ Values for Platelet 5-$HT_{2A}$ Receptor Binding in Healthy Subjects

| | | $B_{max}$ (fmol/mg protein) | | $K_d$ (nM) | |
|---|---|---|---|---|---|
| | *n* | Mean | SD | Median | Interquartile Range |
| Total group | 112 | 23.3 | 6.1 | 0.81 | 0.58–1.21 |
| All males | 39 | 23.2 | 6.2 | 0.77 | 0.51–1.06 |
| All females | 73 | 23.3 | 6.0 | 0.88 | 0.58–1.33 |
| Premenopausal females | 51 | 23.1 | 6.6 | 0.90 | 0.55–1.36 |
| Postmenopausal females | 22 | 23.8 | 4.7 | 0.75 | 0.57–1.35 |

*Note*: None of the differences were significant.

There was a significant positive correlation between $B_{max}$ and $K_d$ values ($r = 0.38$; $p < .001$). There was a slight, but statistically significant, negative correlation between protein concentrations and $B_{max}$ ($r = -0.29$; $p = .002$), but not between protein concentrations and $K_d$ ($r = -0.12$; $p = .22$). ANOVA analyses failed to detect a significant contribution of protein content to any of the relations between other variables.

## Interindividual Variability

$B_{max}$ showed an approximately normal distribution (skewness 0.32) with a mean (± SD) of 23.3 ± 6.1 fmol/mg protein (range 10.5–39.8). $K_d$ was approximately log-normally distributed (skewness 0.19 for log values) with a median of 0.81 nM (range 0.35–2.34). There were no significant differences in $B_{max}$ or $K_d$ between men and women or between premenopausal and postmenopausal women (Table 1), and no association between age and $B_{max}$ or $K_d$ was found by linear regression (Figure 2).

The body weight of the subjects was 67.2 ± 10.6 kg and the body mass index (BMI) was 23.0 ± 2.9 kg/$m^2$ (means ± SD). Body weight and BMI showed a weak, but statistically significant, negative correlation with $K_d$ ($r = -0.19$; $p = .04$, and $r = -0.21$; $p = .03$, respectively), but not with $B_{max}$ ($r = -0.12$; $p = .23$, and $r = -0.10$; $p = .31$, respectively). The systolic blood pressure of the subjects was 129 ± 18 mmHg, and the diastolic blood pressure was 76 ± 8.6 mmHg (means ± SD). There were no correlations between systolic blood pressure and $B_{max}$ ($r = 0.09$; $p = .38$) or $K_d$ ($r = -0.05$; $p = .55$) or between diastolic blood pressure and $B_{max}$ ($r = 0.03$; $p = .74$) or $K_d$ ($r = 0.06$; $p = .51$).

Ten subjects were poor metabolizers of dextromethorphan, and one was a poor metabolizer of mephenytoin. These subjects did not differ from the extensive metabolizers with regard to $B_{max}$ or $K_d$ values. There were no correlations between dextromethorphan (CYP2D6) phenotype ratio and $B_{max}$ ($r = 0.12$; $p = .23$) or $K_d$ ($r = -0.04$; $p = .62$) or between mephenytoin (CYP2C19) phenotype ratio and $B_{max}$ ($r = -0.07$; $p = .47$) or $K_d$ ($r = 0.05$; $p = .56$).

There was a trend toward higher $B_{max}$ and $K_d$ values in samples obtained between 8:00 A.M. and noon than in

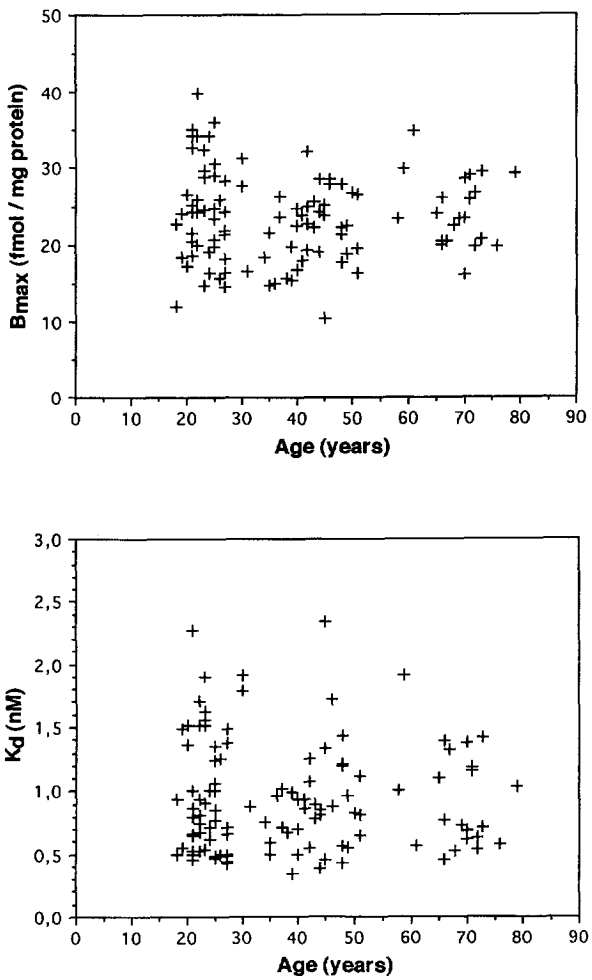


**Figure 2.** Correlation between age and $B_{max}$ (*upper panel*) and age and $K_d$ (*lower panel*) for platelet 5-$HT_{2A}$ receptor binding in 112 healthy subjects. For $B_{max}$: $r = 0.02$, $p = .83$; for $K_d$: $r = -0.04$, $p = .66$.

**Table 2.** Seasonal Variations in Platelet 5-$HT_{2A}$ Receptor Binding in Healthy Subjects

| | | $B_{max}$ (fmol/mg protein) | | $K_d$ (nM) | |
|---|---|---|---|---|---|
| | *n* | Mean | SD | Median | Interquartile Range |
| Summer (June–August) | 21 | 23.9 | 5.6 | 0.96 | 0.83–1.35 |
| Autumn (September–November) | 69 | 22.8 | 5.8 | 0.67* | 0.52–0.94 |
| Winter (December–February) | 22 | 25.0 | 5.2 | 1.16 | 0.76–1.56 |

*$p < .001$ versus summer and winter.

the samples obtained between noon and 4:00 P.M. (23.8 vs. 23.0 fmol/mg protein for $B_{max}$ and 0.98 vs. 0.88 nM for $K_d$), but the differences were not statistically significant. Seasonal variations in $B_{max}$ and $K_d$ are presented in Table 2. $B_{max}$ was significantly lower in samples taken during the light half of the year (April–September) than during the dark half of the year (October–March) (21.7 vs. 25.1 fmol/mg protein; $p = .001$).

### Intraindividual Variability

The intraindividual variability, as studied by one sample weekly for 4 weeks, is presented in Table 3. In general, the coefficient of variation was higher for $K_d$ than for $B_{max}$. For females, the intraindividual variability during the menstrual cycle is illustrated in Figure 3. We found a tendency toward higher $B_{max}$ and $K_d$ in weeks 2 and 3 than in weeks 1 and 4 in the menstrual cycle, with a statistically significant increase in $B_{max}$ from week 1 to week 2 in females not taking contraceptive pills (23.6 vs. 25.3 fmol/mg protein, $p = .03$). $B_{max}$ and $K_d$ was generally somewhat higher among females taking contraceptive pills than among females not taking contraceptive pills, a difference which was statistically significant for $K_d$ at week 1 (1.44 vs. 0.75 nM, $p = .04$) and week 4 (1.19 vs. 0.64 nM, $p = .04$).

## DISCUSSION

The mean $B_{max}$ of 23.3 fmol/mg protein found in the present study is in the lower range of the interval of means reported earlier. Using LSD as radioligand, mean published $B_{max}$ values in healthy subjects have varied between 14.5 (McBride et al. 1987) and 84 (Arora and Meltzer 1993). Our results are similar to the means reported by Norman et al. (1990), McBride et al. (1987, 1994), and Sheline et al. (1995), but lower than those reported by Steckler et al. (1993), Arora and Meltzer (1989a), Geaney et al. (1984), Cowen et al. (1986), Schächter et al. (1985), and Pandey et al. (1990, 1993).

The median $K_d$ of 0.81 nM in the present study is similar to the means reported by Cowen et al. (1986) and Arora and Meltzer (1989a, 1993), but somewhat higher than those found by Schächter et al. (1985), Norman et al. (1990), Steckler et al. (1993), Geaney et al. (1984) and Sheline et al. (1995), and somewhat lower than reported by McBride et al. (1987, 1994). Mean $K_d$ values in healthy subjects in earlier studies have varied between 0.5 nM (Geaney et al. 1984; Norman et al. 1990) and 1.7 nM (McBride et al. 1994).

The observed discrepancies in binding characteristics may be caused by several factors. First, the drugs used to define specific binding vary; most commonly

**Table 3.** Intraindividual Variability in $B_{max}$ and $K_d$ Values for Platelet 5-$HT_{2A}$ Receptor Binding in Males and Females as Studied by One Sample Weekly for 4 Weeks

| | Coefficient of variation (%) and $B_{max}$. Mean (range) | Coefficient of variation (%) and $K_d$. Mean (range) |
|---|---|---|
| Males ($n = 5$) | 7.8 (3.4–11.5)* | 24.0 (7.8–35.8) |
| Females not taking contraceptive pills ($n = 6$) | 10.7 (4.2–15.6)† | 24.1 (3.3–41.0) |
| Females taking contraceptive pills ($n = 6$) | 10.5 (1.8–19.8)* | 25.6 (12.5–38.7) |

*$p = .04$ versus coefficient of variation for $K_d$.
†$p = .06$ versus coefficient of variation for $K_d$.

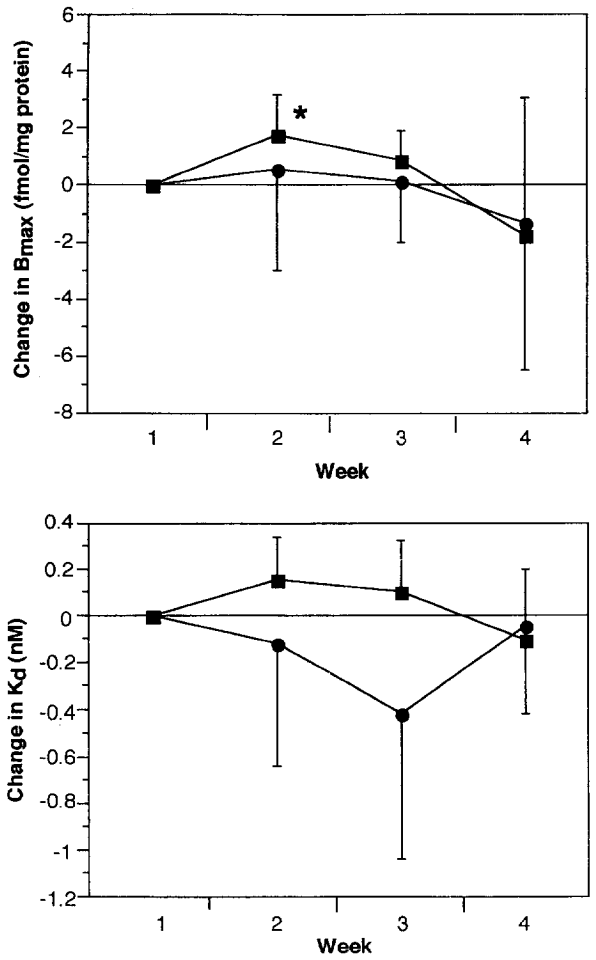


**Figure 3.** Changes in $B_{max}$ (*upper panel*) and $K_d$ (*lower panel*) for platelet 5-$HT_{2A}$ receptor binding during the menstrual cycle in six drug-free females (*squares*) and six females taking contraceptive pills (*circles*), relative to week 1 in the menstrual cycle. Mean ± SD. *$p$ = .03 compared with week 1.

spiperone (300 nM), and ketanserin (1.0 μM) have been used. Second, both fresh platelet membranes and frozen platelet membranes have been used, and the procedures for preparation of the platelet tissue is not consistent across studies. Third, differences in the homogenization and washing procedures may result in a differing ratio of the receptor-bearing membrane fragments to other proteinaceous constituents of the suspension. Fourth, the rate and duration of filtration during tissue harvesting may affect the amount of ligand displaced from the receptor as well as "nonspecific" sites. Finally, insufficient washing procedures, with EDTA left in the sample for protein quantification, may give falsely reduced protein contents and, consequently, falsely enhanced $B_{max}$ values when the Lowry method is used (Lowry et al. 1951). EDTA, even at concentrations as low as 0.5 mM, interfere with protein determinations according to Lowry (Ji 1973). We have used the modified protein content method described by Markwell et al. (1978), thus eliminating this pitfall.

We found that $B_{max}$, but not $K_d$, correlated negatively to the protein content in our experiments. This correlation was, however, exclusively caused by six outliers who had a protein content below 0.52 mg/ml and who were the only with $B_{max}$ values higher than 34 fmol/mg protein. Our findings are apparently in contrast to the results reported by Steckler et al. (1993), where protein concentrations of 0.3 to 0.4 mg/ml gave relatively stable $B_{max}$ and $K_d$ values, whereas $B_{max}$ was reduced when the protein content was less than 0.25 mg/ml. As we used half the incubation volumes used by Steckler et al. (1993), the total protein content per well has been approximately the same. Further investigations are obviously needed to clarify the influence of the content or the concentration of protein on platelet 5-$HT_{2A}$ receptor binding.

In the present study, we have demonstrated that gender and age are not likely to have a significant effect on [$^3$H]-LSD binding. The lack of gender effects is in accordance with the results reported by Cowen et al. (1986), McBride et al. (1987), Biegon et al. (1987), Pandey et al. (1990, 1993), Andres et al. (1993), and Pandey et al. (1995), but contrary to those reported by Arora and Meltzer (1989a), who reported a higher mean $B_{max}$ in males than in females. Our findings of similar values for $B_{max}$ and $K_d$ in all adult age groups are in agreement with the studies of Arora and Meltzer (1989a, 1993), Pandey et al. (1990, 1993), McBride et al. (1987), and Pandey et al. (1995), but in disagreement with the studies of Geaney et al. (1990) and Norman et al. (1990), in which a decrease in $K_d$ with increasing age was found, and the study of McBride et al. (1994), in which a decrease in both $B_{max}$ and $K_d$ with increasing age was found. The reason for the tendency toward lower $K_d$ and $B_{max}$ with increasing age in some studies is not known, but the number of subjects included in these studies is generally low (15–45 individuals). Although age-related changes have been observed for a variety of enzymes and receptors, others have been unable to show any age effect on other serotonin variables (Halbreich et al. 1991).

There is extensive evidence that serotonin and serotonin receptors play an important role in the regulation of appetite and food intake and that serotonin reduces the intake of carbohydrates through its effect in the hypothalamus (Blundell 1984). Arora and Meltzer (1989a) found a significant negative correlation between $K_d$ for 5-$HT_{2A}$ receptors and weight loss ($r = -0.47$) in 18 depressed patients, whereas Goodwin et al. (1987) found a significant increase in $B_{max}$ and $K_d$ in 23 healthy subjects after 3 weeks of dieting. The results from the present study cannot be compared directly with those from these studies, as we have studied a primarily nondieting population. Further studies are therefore needed to

elucidate the role of 5-$HT_{2A}$ receptors in body weight regulation and dieting.

Serotonin has been implicated in blood pressure regulation (Kamal et al. 1984), and the antihypertensive agent ketanserin is a 5-$HT_{2A}$ receptor antagonist (Amstein et al. 1989), although it also exerts $\alpha_1$-adrenergic antagonism. Studies on 5-$HT_{2A}$ receptor binding in hypertensive patients have not been performed, but other serotonin parameters, such as platelet serotonin uptake, content, and release, as well as serotonin-induced shape change of platelets, have been found to be altered (Kamal et al. 1984; Amstein et al. 1989). In the present study, we were unable to find any correlation between $B_{max}$ or $K_d$ for 5-$HT_{2A}$ receptor binding and blood pressure, which however does not exclude that such a correlation may exist when hypertensive subjects are also included.

Although our results indicate that seasonal variations in $B_{max}$ and $K_d$ may exist, the present study is incomplete with this respect. A longitudinal intraindividual study over 1 year has been carried out in our laboratory, and preliminary results from this study also indicate that seasonal variations exist, with fluctuations consistent with the results from the present study. Time of day has been suggested to be an important variable in the assessment of platelets serotonin uptake (Rausch et al. 1982), but a clear pattern has not been apparent (Halbreich et al. 1991). Diurnal variations in 5-$HT_{2A}$ receptor parameters cannot be completely ruled out on basis of our study, as only the 8-hour interval from 8:00 A.M. to 4:00 P.M. is studied.

Studies on the effect of sex steroids on platelet serotonin variables are conflicting. Poirer et al. (1986) found no changes in imipramine binding during the menstrual cycle, whereas Weizman et al. (1988) reported upregulation of platelet imipramine binding in females taking contraceptive pills. The results of Best et al. (1989) suggest that estrogen alone is insufficient to change platelet imipramine binding or 5-$HT_{2A}$ receptor binding. On the other hand, treatment with estradiol significantly increases the number of 5-$HT_{2A}$ receptors in the rat brain (Sumner and Fink 1995). We found a minor upregulation of 5-$HT_{2A}$ receptors in week 2 compared with week 1 of the menstrual cycle. Although $B_{max}$ and $K_d$ generally were somewhat higher throughout the menstrual cycle among females taking contraceptive pills than among females not taking contraceptive pills, we found no significant overall effect. There is, however, a risk that the present study was too small to detect real differences in this respect.

In conclusion, this study in healthy adults implicates that age and gender have no effect of platelet 5-$HT_{2A}$ receptor parameters, that body weight and body mass index show a negative correlation to $K_d$, and that blood pressure, CYP2D6 phenotype, and CYP2C19 phenotype are not correlated to $B_{max}$ or $K_d$. Diurnal variations were not observed in our samples, obtained between 8:00 A.M. and 4:00 P.M. Seasonal variations may exist, but additional studies are required to extend our observations. In females, there were significant changes in $B_{max}$ during the menstrual cycle, and treatment with contraceptive pills had minor effects on $K_d$. These factors should be taken into account when platelet 5-$HT_{2A}$ receptor binding studies are performed in patients with neuropsychiatric disorders.

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