Psychopharmacology (2003) 166:221–227
DOI 10.1007/s00213-002-1353-2

ORIGINAL INVESTIGATION

Ashwin A. Patkar · Raman Gopalakrishnan · Wade H. Berrettini · Stephen P. Weinstein · Michael J. Vergare · Frank T. Leone

Differences in platelet serotonin transporter sites between African-American tobacco smokers and non-smokers

Received: 12 April 2002 / Accepted: 14 November 2002 / Published online: 13 February 2003
© Springer-Verlag 2003

Abstract *Rationale:* The serotonin transporter (5HTT) regulates the magnitude and duration of serotonergic neurotransmission. Although nicotine and other constituents of tobacco smoke may influence serotonin turnover among animals, few studies have examined whether smoking is associated with alteration in 5HTT in humans. *Objective:* We investigated whether tobacco smokers and non-smokers differed in platelet tritiated paroxetine binding, a measure of 5HTT sites, and whether severity of nicotine dependence (ND) was related to 5HTT measures. *Methods:* Tritiated paroxetine binding sites on platelets were assayed in 26 African-American smokers and 30 non-smokers. Severity of smoking was assessed using the Fagerstrom Test for Nicotine Dependence (FTND). Relationships between FTND scores and maximum number of transporter sites (B_{max}) and affinity constant (K_d) of paroxetine binding were determined. *Results:* B_{max} values showed a significant negative correlation with FTND scores (rho=–0.28, $P<0.01$). Notably, smokers with higher ND had significantly lower B_{max} compared to those with lower ND and non-smokers; the latter two groups did not differ in B_{max} ($F=3.92$, $P<0.05$). Smokers scored higher on impulsivity than non-smokers, however, behavioral variables did not influence the relationship of smoking with B_{max}. Age, gender and K_d values were not associated with smoking or B_{max}.*Conclusions:* Smoking, in particular higher nicotine dependence, appears to be correlated with decreased density of platelet 5HTT sites in African-Americans. The nature of the relationship and whether similar changes occur in the brain merit further investigation.

Keywords Smoking · Nicotine · Serotonin · Tobacco · Serotonin transporter

A. A. Patkar (✉) · R. Gopalakrishnan · S. P. Weinstein · M. J. Vergare
Department of Psychiatry and Human Behavior, Thomas Jefferson University, Philadelphia, PA 19107, USA
e-mail: ashwin.patkar@mail.tju.edu
Tel.: +1-215-9552542
Fax: +1-215-5032850

W. H. Berrettini
Department of Psychiatry, Center for Neurobiology and Behavior, University of Pennsylvania, Philadelphia, PA 19104, USA

F. T. Leone
Department of Medicine, Division of Pulmonology and Critical Care, Thomas Jefferson University, Philadelphia, PA 19107, USA

Introduction

The high prevalence of smoking, its associated health risks and the limited pharmacological interventions available for smoking cessation highlight the importance of understanding the biological mechanisms that contribute to smoking. Although environmental factors such as peer influences may affect smoking behavior, a significant determinant of continued tobacco use is addiction to nicotine, the principal psychoactive constituent of tobacco smoke (Dani and Heinemann 1996). There is increasing evidence that in addition to the dopamine system, serotonergic mechanisms may mediate the behavioral effects of nicotine. Animal experiments have found that administration of nicotine inhibits serotonin uptake and stimulates release of serotonin in the brain (Benwell and Belfour 1982; Ribeiro et al. 1993; Reuben and Clarke 2000). The nicotine-serotonergic interactions in different parts of the brain have been proposed to mediate distinct behavioral effects of nicotine (Seth et al. 2002). Animal models investigating the effects of pharmacological manipulations of brain serotonin have been consistent with these findings (Olausson et al. 2002). For example, enhancing serotonergic neurotransmission by fluoxetine, a selective serotonin reuptake inhibitor, reversed the reward deficits observed in nicotine withdrawal (Harrison et al. 2001).

Compared to the preclinical evidence, human studies examining the relationship between nicotine use and serotonergic function have been limited, and the findings have been less robust. On the one hand, in vitro studies have found that nicotine administration may stimulate

serotonin release and block serotonin uptake in human platelets (Rausch et al. 1989). Also, smokers have been found to exhibit a blunted prolactin response to fenfluramine compared to controls, indicating that neuroendocrine measures of central serotonergic activity may differ between smokers and non-smokers (Anthenelli and Maxwell 2000). In contrast, neuroimaging studies have failed to demonstrate any differences in serotonin transporter availability between smokers and non-smokers). The conflicting findings may be related to several clinical variables that are difficult to control among humans. For example, conditions such as depression, impulsive behaviors and substance abuse that are often prevalent in tobacco smokers may confound the relationship between serotonergic function and smoking (Linnoila and Virkunnen 1992; Coccaro et al. 1996; Buydens-Branchey et al. 1997). Despite the potential methodological difficulties, the sparse human data indicates the need for further studies in this area.

Various lines of evidence suggest that the serotonin transporter (5HTT) may be an attractive candidate to study serotonin function in smokers. The 5HTT regulates the magnitude and duration of serotonergic neurotransmission and represents an initial target site for certain antidepressants (Graham and Langer 1992). Animal studies have demonstrated that chronic exposure to nicotine is associated with a reduction in 5HTT sites in the brain (Xu et al. 2001). Genetic studies also support a role for 5HTT in nicotine dependence. Heils et al. (1996) reported a polymorphism in the 5' promotor region of the 5-HTT gene, yielding a short (S) and a long (L) variant of the allele. An association between the L allele and smoking has been reported in some studies (Arinami et al. 1999; Ishikawa et al. 1999). Indirect evidence implicating the 5HTT in nicotine dependence comes from studies of substance abusing individuals. Changes in platelet tritiated imipramine or paroxetine binding have been reported in alcoholics (Patkar et al. 1995), heroin abusers (Macedo et al. 1995), and cocaine abusers (Patkar et al. 2003). Furthermore, in addition to nicotine, other constituents of tobacco smoke may also be associated with altered serotonergic function. Smokers have been found to have reduced levels of monoamine oxidase (MAO) enzyme activity in the brain, which in turn may lead to elevated catecholamine levels and a compensatory downregulation of serotonin transporter (Fowler et al. 1996; Mendez-Alvarez et al. 1997).

Surprisingly, there have been very few studies of smokers that involved platelet serotonin markers. The goal of the present study was to investigate platelet measures of serotonin function in smokers. Based on preclinical data, we postulated that platelet 5HTT, a peripheral measure of serotonin activity, would differ between smokers and non-smokers and that severity of nicotine dependence would be related to alterations in 5HTT levels. Since behavioral measures of impulsivity and aggression may be related to platelet 5HTT levels (Coccaro et al. 1996; Patkar et al. 2003), we also assessed these measures in our sample. We selected a peripheral measure of serotonin activity, since there are methodological difficulties in studying central serotonin function in humans. Although platelets constitute a peripheral site, the serotonin transporter sites on the platelet have been shown to be structurally and biologically similar to the corresponding sites in the human brain (Da Prada et al. 1988). In this study, we selected tritiated paroxetine, a widely used radioligand to label the platelet serotonin transporter sites.

Materials and methods

Subjects

Twenty-six tobacco smokers were recruited from those responding to local advertisements in Philadelphia. This study was part of a project that examined serotonergic function among African-American population; therefore, the sample included only African-American subjects. The protocol was approved by the Committee on Protection of Human Subjects and following a description of the study, informed consent was obtained. The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al. 1997) was then administered to individuals who volunteered for the study. Individuals who fulfilled criteria for nicotine dependence were included in the study, while those with a diagnosis of other substance abuse or dependence, schizophrenia, major depression, dysthymic disorder, anxiety disorders, bipolar disorder, schizoaffective disorder, a serious medical illness or pregnancy or those receiving psychotropic medications were excluded. Nine patients were excluded for axis I diagnoses (three major depression, two dysthymic disorder, two substance abuse, two anxiety disorders). One patient with severe COPD was also excluded. Among subjects included in the study, four patients had lifetime axis I diagnoses (two had dysthymic disorders, one had generalized anxiety disorder and one had alcohol abuse). The presence of cotinine in urine was confirmed by Smoke Check Test (One-Step Detect Associates, Jefferson Hills, Pa., USA), a one-step immunoassay for qualitative detection of cotinine in human urine at a cut-off of 200 ng/ml. Urine drug screens were obtained for all subjects by Accutests (Jant Pharmacal Corporation, Calif., USA). The Accutest Multi-Drug Screen is a one-step immunoassay for the detection of cocaine, opiates, tetrahydrocannabinol, amphetamines and phencyclidine in the urine.

Thirty African-American controls were recruited though local advertisements. Consent and screening procedures were similar to those followed for smokers. Control subjects were excluded if they were tobacco users or had a history of alcohol or illicit drug abuse or dependence, a major psychiatric disorder (schizophrenia, major depression or bipolar disorder), a positive urine drug screen or were taking psychotropic medications.

Assessments

All subjects and controls had their medical health documented by medical history and physical examination. Smoking status was rated on the Fagerstrom Test for Nicotine Dependence (FTND), a widely used and validated six-item questionnaire to assess severity of smoking (Heatherton et al. 1991). This is a revised version of the Fagerstrom Tolerance Questionnaire (FTQ) (Fagerstrom 1978) and can be completed in less than 5 min. The six questions on the FTND pertain to: smoking within 30 min of waking up, difficulty in stopping smoking in places where it is forbidden, difficulty in giving up morning cigarettes, number of cigarettes smoked per day, smoking during early part of the day and smoking despite being ill. The total scores on the FTND range from 0 to 10. Non-smokers were defined as individuals who had smoked less than 100

cigarettes in their lifetime and who tested negative for urinary cotinine.

Subjects and controls then completed additional behavioral assessments. These included the Buss-Durkee Hostility Inventory (BDHI) (Buss and Durkee 1957), the Barratt Impulsivity Scale (BIS), and the Beck Depression Inventory (BDI) (Beck and Steer 1987). The BDHI is a widely used 75-item true-false questionnaire which takes about 20 min to complete. It is composed of two main factors (motor aggression and hostility) and eight subscales. The motor aggression factor is composed of four subscales (direct, indirect, verbal and irritability), while the hostility factor is composed of two subscales (resentment and suspiciousness). The remaining subscales measure guilt and negativism. Reliability and validity have been established for the instrument. The BIS is another well-known 15-min, 34-item, self-report questionnaire to measure different dimensions of impulsivity. It contains three subscales that measure motor, cognitive and non-planning impulsivity. The BDI is a 21-item self-report questionnaire that assesses depressive symptomatology experienced during the previous week; it takes about 10 min to complete. The BDI has been widely employed in clinical settings and has been shown to have high test-retest reliability and item validity.

Paroxetine binding assay

Subjects were instructed to fast overnight and blood draws were performed in the morning. Smokers had their last cigarette within 6 h of the blood draw. Women were studied in the initial follicular phase of the menstrual cycle. This phase was defined clinically as the 10-day period following the end of the menstrual phase. Twenty ml of venous blood was collected in ethylendiaminetetraacetic acid (EDTA) containing tubes at room temperature and processed within 4 h to harvest platelets. The platelet pellets were immediately frozen at −80°C until they were assayed. Paroxetine binding was performed using the technique described by Ozaki et al. (1994), with minor modifications. Briefly, platelet membranes were homogenized in assay buffer by ultrasonification. After washing and centrifugation, the platelet membranes were resuspended in assay buffer (120 mM sodium chloride, 50 mM TRIS hydrochloride and 5 mM potassium chloride, pH 7.5). Protein concentrations were determined by the method of Lowry et al. (1951). Specific binding of tritiated paroxetine ([^{3}H]paroxetine) (Life Science Products Inc., Boston, Mass., USA) was determined at six different concentrations in the presence and absence of 1 μmol/l of fluoxetine (Sigma, St Louis, Mo., USA). The incubation mixture consisted of 10–20 μg membrane protein, 10 μl buffer or fluoxetine and 5 μl [^{3}H]paroxetine in 2 ml volume. After incubation at room temperature for 90 min, 5 ml ice-cold buffer was added to stop the reaction. The samples were filtered through premoistened Whatman filters. Filters were rinsed twice with ice-cold buffer, dried, added to vials containing scintillation fluid and counted for radioactivity in a scintillation counter. The data was transformed using computer programs to determine transporter densities (B_{max}) and affinity constant (K_d). The B_{max} of paroxetine binding was expressed as fentomol/mg while the K_d was expressed as nanomol/l. The laboratory personnel were blind to the clinical data. The assessment procedure including the blood draw took about 2.5 h.

Statistical analyses

The primary biological variables were B_{max} and K_d of paroxetine binding. The primary behavioral measure was the total FTND score. Other behavioral measure included scores on BDI, BIS and BDHI. Comparisons between smokers and non-smokers were performed using one-tailed *t*-tests for continuous variables and chi-square tests for categorical variables. Bonferroni correction was used for multiple comparisons. Subsequently smokers were divided into higher and lower severity of nicotine dependence based on a FTND score cutoff of 6, which corresponded to about one standard deviation above the mean score. Analysis of variance (ANOVA) was employed to compare the two with each other and with controls with respect to paroxetine binding. When appropriate, post-hoc Scheffe tests were conducted to examine significant ANOVA effects. Correlations between scores on behavioral and biological variables were performed using product moment (Pearson) or rank order (Spearman) correlations as appropriate.

Results

Subjects

Data on 56 African-American individuals are reported: 26 were current tobacco smokers and 30 were non-smoking controls. Six former smokers who had quit periods ranging from 4 months to 14 years and two individuals whose blood samples could not be assayed for technical reasons were not included in the study. The smokers (69.8% male, age=32.50±5.16 years, 44.3% single, 38.4% unemployed) and controls (53.3% male, age= 30.3±6.05 years, 49.6% single, 26.8% unemployed) did not differ significantly in gender, age, marital or employment status (all χ^2<2.5, *df*=1; *t*=0.35, *df*=54, *P*>0.05 in each case). On average, subjects smoked 16 cigarettes daily and had been smoking for 17 years. The mean FTND score was 4.23±1.68 with a range of 2 to 8, and about 34% of smokers had FTND scores of 6 or greater. All smokers tested positive for urinary cotinine. Age or gender was not related to FTND scores. Clinically no subject reported being in nicotine withdrawal.

Tritiated paroxetine binding and behavioral variables among tobacco smokers and controls

As summarized in Table 1, tobacco smokers had significantly lower B_{max} values compared to controls (*t*=2.02, one tailed, *P*<0.01; *P*<0.05 after Bonferroni correction). However, there was no significant difference in K_d values between smokers and controls and the correlation between B_{max} and K_d values was not statistically significant (*r*=0.16, *P*=0.21). Smokers also exhibited significantly higher total scores on the Barratt Impulsivity Scale (BIS) compared to controls; the between-group differences across subscale scores of the BIS were consistent with comparisons for the total BIS score. No significant between-group differences were observed for measures of depression (BDI) and hostility (BDHI). The platelet counts did not differ significantly between smokers (212±32 B/L) and control subjects (238±48 B/L).

Relationship between nicotine dependence and tritiated paroxetine binding

We then analyzed the data to determine whether level of nicotine dependence was related to B_{max} values. A significant negative correlation was observed between FTND scores and B_{max} values (rho=–0.28, *P*<0.01, one

Table 1 Tritiated paroxetine binding and behavioral measures in tobacco smokers and controls

Assessment measures	Subjects (n=56)		t
	Smokers (n=26) mean±SD	Controls (n=30) mean±SD	
Paroxetine binding			
B_{max}	841.88±208.22	963.61±237.67	2.02*
K_d	0.25±0.08	0.27±0.07	1.17
Barrat impulsivity(BIS)			
Motor	9.88±2.26	8.36±2.41	1.92
Cognitive	10.73±3.62	8.86±3.14	1.87
Non-planning	12.38±3.96	10.03±4.01	1.92
Total score	33.00±8.65	27.26±9.96	2.03*
Buss Durkee (BDHI)			
Assault	2.46±0.76	2.30±0.92	0.71
Guilt	2.34±0.84	2.13±0.81	0.96
Indirect aggression	2.76±0.81	2.73±0.90	0.15
Verbal aggression	3.42±1.13	3.16±0.87	0.95
Irritability	2.73±0.72	2.70±0.79	0.15
Negativism	2.38±0.75	2.33±0.99	0.21
Resentment	2.63±0.99	2.80±0.93	0.67
Suspicion	2.84±0.96	2.70±0.96	0.52
Total score	18.34±4.11	17.53±5.43	0.62
Beck Depression (BDI)	6.33±3.64	5.60±3.0	0.70

*P<0.01, df=54, one-tailed t-tests

tailed; P<0.05 after Bonferroni correction) The findings are summarized in Fig. 1.

Since smokers were more impulsive than controls, we explored and found a weak correlation of B_{max} values with the BIS total score (r=0.21, P=0.08) and the BIS motor subscale score (r=0.22, P=0.07). However, B_{max} did not show a significant correlation with the BDHI total score (r=–0.10) and the BDI score (r=–0.12). We also examined the relationship between B_{max} and FTND scores controlling for BDI, BIS and BDHI scores using tests of partial correlation. The negative correlation between B_{max} and FTND scores described above continued to remain significant after covarying for BDI ($r_{partial}$=–0.28, P<0.01), BIS ($r_{partial}$=–0.29, P<0.01) and BDHI scores ($r_{partial}$=–0.29, P<0.01). Also, no significant relationships were observed between B_{max} values and demographic characteristics, including age (r=0.13), gender (χ^2=0.48), employment (χ^2=0.31), and marital status (χ^2=0.79).

To determine whether more severely nicotine dependent smokers differ from the less severely dependent individuals in terms of B_{max} of paroxetine binding, we examined the distribution of FTND scores in smokers and established a cutoff of one standard deviation above the mean that corresponded to a FTND score of approximately 6. The mean (4.2) and median (4.6) did not differ widely. Nine (34.61%) smokers had FTND scores of ≥6. Almost all smoked more than 20 cigarettes per day, and over 75% smoked their first cigarette within 30 min of waking up. A comparison of B_{max} values between smokers with higher and lower nicotine dependence and controls found a significant differences between the three groups [ANOVA, F(2,54)=3.91, P=0.02]. Post-hoc Scheffe tests revealed that smokers with FTND scores ≥6 had significantly lower B_{max} (730.50±115.38) than those with FTND scores <6 (900.85±224.54) (mean difference=170.34, SE=86.21, P<0.05) and controls (963.61±237.67) (mean difference=233.09, SE=83.38, P<0.05); the latter two groups did not differ from each other (mean difference=62.75, SE=66.60, P=0.64). The relationship of lower B_{max} scores with the higher nicotine dependence category is consistent with the inverse correlation observed between B_{max} and FTND scores. The results are summarized in Fig. 2.

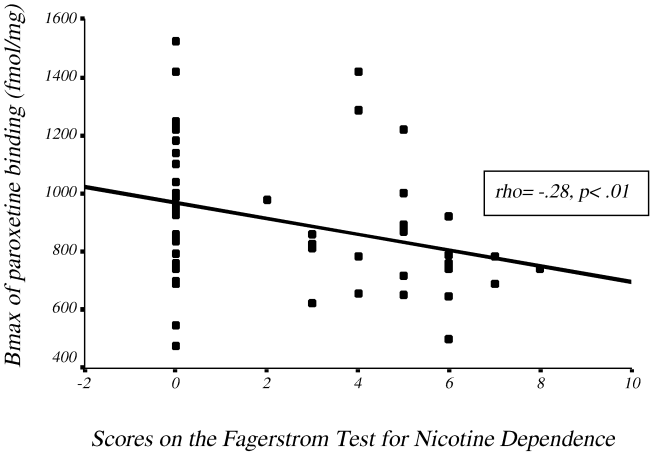

Fig. 1 Correlation of B_{max} values scores on the Fagerstorm

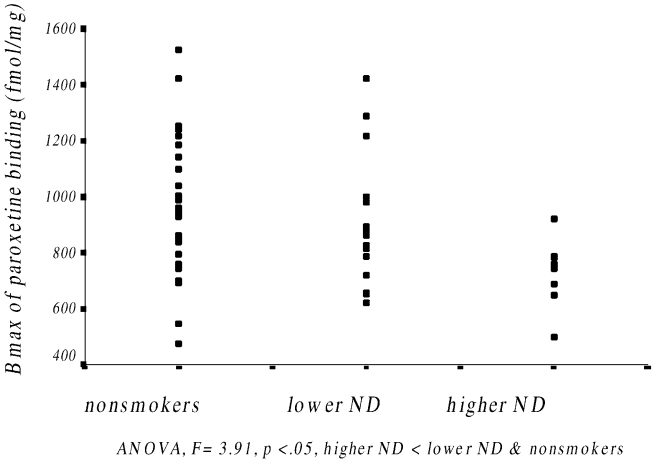

Fig. 2 Distribution of B_{max} among non-smokers and smokers with higher and lower nicotine dependence (ND)

Discussion

Serotonin transporter (5HTT) and tobacco smoking

The present study has two main findings of interest. First, a significant inverse correlation was observed between densities of platelet 5HTT sites and scores on the Fagerstrom Test for nicotine dependence (FTND). Second, densities of platelet 5HTT sites were significantly lower only in smokers with higher nicotine dependence compared to non-smokers; those with lower nicotine dependence did not differ from non-smokers with respect to 5HTT. Self-reports of smoking were confirmed by urinary cotinine, there were no significant demographic differences between smokers and non-smokers, and effects of potentially confounding behavioral variables such as depression were controlled. Moreover, the FTND and its earlier version, the Fagerstrom Tolerance Questionnaire, have been found to correlate with cotinine and carbon monoxide concentrations in blood and urine (Pomerleau et al. 1990; Etter and Perneger 2001). Given these methodological issues, the findings indicate that high level of nicotine dependence may be associated with platelet 5HTT densities in humans.

There appear to be two principal pathways for smoking to be associated with alterations of 5HTT. First, in vitro studies have found that exposure to nicotine, the principal constituent of tobacco smoke, is associated with increase in serotonin release and inhibition of serotonin uptake in human platelets (Rausch et al. 1989). Also, reduced paroxetine binding sites have been found in brains of rats exposed to nicotine at doses that replicated plasma cotinine levels in human smokers (Xu et al. 2001). Second, tobacco smoke is found to inhibit partially the enzyme monoamine oxidase (MAO) in human platelets and brain (Berlin et al. 1995; Fowler et al. 1996). Since MAO metabolizes amines such as serotonin, it is possible that long-term inhibition of MAO by tobacco smoking may enhance serotonin concentration and lead to changes in transporter function. However, it is also possible that the 5HTT differences preceded tobacco use and be a trait marker for smoking; some genetic studies support this hypothesis (Arinami et al. 1999). It was beyond the scope of this study to determine whether the causality between 5HTT changes and smoking and longitudinal studies may be required to answer this question.

A few other findings deserve comment. First, contrary to the literature we failed to observe an inverse correlation of B_{max} with BIS or BDHI scores. This may be because the scores on these variables were low with a restricted range of values. Also, the sample size was modest and it is possible that the relationships could have reached significance with a larger sample [for example, the correlation of B_{max} with BIS tending toward significance in smokers (n=26)]. Second, it is worth noting that the B_{max} of platelet paroxetine binding in our African-American sample (907±231 fmol/mg) was lower than that reported in some studies of primarily Caucasian subjects in the US (1024±511) (Coccaro et al. 1996) and Europe (1237±182) (Strachan and Maughan 1998). This raises the intriguing question whether there could be ethnic differences in platelet serotonin transporter sites. However even lower B_{max} values than those observed in our sample have been reported in other studies of healthy volunteers of Caucasian background (Maguire et al. 1995; Greenberg et al. 1999). Studies comparing large samples of different ethnic groups may clarify this issue.

Limitations

The findings must be interpreted in light of certain methodological limitations. The study recruited African-American individuals only and the results may not be generalizable to smokers from other ethnic backgrounds. Biological studies employing ethnically matched samples of subjects and controls seem to be particularly relevant to tobacco smoking because metabolism of nicotine may differ across ethnic groups. For example, African-Americans have been reported to exhibit higher blood levels of cotinine compared to Caucasians (Wagenknecht et al. 1990). Nevertheless, biological data on African American tobacco smokers is relatively limited and in this context, the study may contribute to the literature. Second, the FTND has been correlated with cotinine levels primarily in heavy smokers and there are some suggestions that it may not be highly sensitive in light smokers (Etter et al. 1999). While we utilized structured interviews and qualitative urinary cotinine (present or absent) to diagnose and assess smoking status, quantitative estimates of urinary cotinine were not performed. It was therefore not possible to correlate urinary cotinine with B_{max}. We felt that it was important to include women smokers, given the scarcity of similar data in the literature. However the inclusion of women could be criticized due to the potential variability in platelet characteristics with menstruation (Corash et al. 1994). We attempted to control for these effects by examining paroxetine binding

in the same phase of the menstrual cycle. It is also possible that platelet 5HTT may not mirror 5HTT sites in the brain even though the same gene encodes for the 5HTT on platelets and neurons (Lesch et al. 1993). Finally, while we excluded current axis I disorders, axis II psychopathology was not assessed and excluded; this could have confounded the findings.

Clinical implications and conclusions

Since the strength of the inverse correlation between B_{max} and level of nicotine dependence was modest, and the sample size was small, the findings should be considered preliminary and need replication, preferably in a larger sample. Nevertheless, one could postulate the possible implications of decreased B_{max} extrapolating findings from studies in depression that have reported reduced platelet 5HTT densities. The question whether the reductions in platelet 5HTT densities represent trait or state phenomena remains unsettled, with evidence supporting both sides (Sheline et al. 1995). Although the literature is not fully consistent, chronic administration of SSRI has been associated with a reduction in the platelet 5HTT binding sites (reviewed by Blakely et al. 1997). In the brain reduced presynaptic 5HTT may be associated with decrease clearance of extracellular serotonin and elevated serotonin concentration at the receptor sites (Maguire et al. 1993). The data regarding the relationship between platelet 5HTT binding characteristics and response to SSRI treatment is conflicting. On the one hand, decreased expression of platelet 5HTT have been correlated with poor response to SSRI as well as placebo in depressed patients (Sheline et al. 1995). Consistent with these findings, two large double-blind, placebo-controlled clinical trials investigating the efficacy of SSRIs as smoking cessation treatments reported negative results (Covey et al. 2002; Niaura et al. 2002). In contrast, depressed patients with lower platelet 5HTT binding at baseline were found to respond better to fluoxetine in some studies (Castrogiovanni et al. 1995). In this context, SSRIs have been reported to help subgroups of smokers, possibly those who are compliant with their medications (Hitsman et al. 1999; Killen et al. 2000). Whether the pretreatment status of 5HTT binding sites influences response to serotonergic agents in smokers seems to merit further investigation. Such a line of research may help eventually to identify subgroups of smokers based on pharmacological characteristics who may be more likely to benefit from different types of serotonergic agents. Our findings also suggest that researchers investigating serotonin transporter function in psychiatric and substance abuse disorders may need to examine and control for the potential confounding influence of tobacco smoking. Finally, we observed that smokers were more impulsive than non-smokers, and there was a trend towards association of impulsivity with B_{max} values. Research exploring the clinical variables that may be associated with changes in B_{max} among smokers seems timely.

In conclusion, the findings indicate that chronic exposure to nicotine may affect serotonin function in humans. Studying whether these changes mediate the reinforcing effects of nicotine may lead to better understanding of the addictive nature of tobacco smoking and may offer new approaches for treatment.

Acknowledgements This research was supported by grant KO8DA00340-02 from the National Institute on Drug Abuse and performed under the auspices of the Commonwealth Office of Drug and Alcohol Programs and the Philadelphia Department of Public Health, Coordinating Bureau for Drug and Alcohol Abuse Programs. We appreciate the contributions of Cheryl Marshall, Heather Murray and Allan Lundy in data collection and analysis.

References

Anthenelli RM, Maxwell RA (2000) Cigarette smoking decreases the prolactin response to serotonergic stimulation in subgroups of alcoholics and controls. Alcohol Clin Exp Res 24:987–995

Arinami T, Obtsuki T, Yamakawa-Kobayashi K, Amemiya H, Fujiwars H, Kawata KI, Ishiguro H, Hamaguchi H (1999) A synergistic effect of serotonin transporter gene polymorphism and smoking in association with CHD. Thromb Haemost 81:853–856

Beck AT, Steer RA (1987) Beck Depression Inventory. Psychological Corporation, Harcourt, Brace, Jovanovich, San Antonio, Texas

Benwell M, Balfour D (1982) Effects of nicotine administration on the uptake and biosynthesis of 5-HT in rat brain synaptosomes. Eur J Pharmacol 84:71–77

Berlin I, Said S, Spreux-Varoquaux O, Olivare R, Launay JM, Puech AJ (1995) Monoamine oxidase A and B activities in heavy smokers. Biol Psychiatry 38:756–61

Blakely RD, Ramamoorthy S, Qian Y, Schroeter S, Bradley CC (1997) Regulation of antidepressant-sensitive serotonin transporters. In: Reith MEA (ed) Neurotransmitter transporters: structure, function, and regulation. Humana Press, New Jersey, pp 29–72

Buss AH, Durkee A (1957) An inventory for assessing different kinds of hostility. J Consult Psychol 21:343–348

Buydens-Branchey L, Branchey M, Fergeson P, Hudson J, McKernin C (1997) The meta-chlorophenylpiperazine challenge test in cocaine addicts: hormonal and psychological responses. Biol Psychiatry 41:1071–1086

Castrigiovanni P, DiMuro A, Marazzati D (1995) Imipramine binding as a predictor of fluoxetine response in depressed patients. Neuropsychobiology 31:64–67

Coccaro EF, Kavoussi RJ, Sheline YL, Lish JD, Csernansky JG (1996) Impulsive aggression in personality disorder correlates with tritiated paroxetine binding in the platelet. Arch Gen Psychiatry 53:531–536

Corash L, Costa JL, Shafer B, Donlon JA, Murphy D (1984) Heterogeneity of human whole blood platelet subpopulations. III. Density-dependent differences in subcellular constituents. Blood 64:185–193

Covey LS, Glassman AH, Stetner F, Rivelli S, Stage K (2002) A randomized trial of sertraline as a cessation aid for smokers with a history of major depression. Am J Psychiatry 159:1731–1737

Dani JA, Heinemann S (1996) Molecular and cellular aspects of nicotine abuse. Neuron 16:905–908

Da Prada M, Cesura AM, Launay JM, Richards JG (1988) Platelets as models for neurons? Experientia 44:115–126

Etter JF, Perneger TV (2001) Measurement of self reported active exposure to cigarette smoke. J Epidemiol Commun Health 55:674–680

Etter JF, Duc TV, Perneger TV (1999) Validity of the Fagerstrom test for nicotine dependence and of the Heaviness of Smoking Index among relatively light smokers. Addiction 94:269–281

Fagerstrom KO (1978) Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict Behav 2:235–241

First MB, Spitzer RL, Gibbon M, Williams JBW (1997) Structured Clinical Interview for DSM-IV disorders (SCID-IV). American Psychiatric Association, Washington D.C.

Fowler J, Volkow N, Wang G, Pappas N, Logan J, MacGregor R, Alexoff D, Shea C, Schlyer D, Wolf A, Warner D, Zezulkova I, Cilento R (1996) Inhibition of monoamine oxidase in the brains of smokers. Nature 379:733–736

Graham D, Langer SZ (1992) Advances in sodium-ion coupled biogenic amine transporters. Life Sci 51:631–645

Greenberg BD, Tolliver TJ, Huang S, Li Q, Bengel D, Murphy DL (1999) Genetic variation in the serotonin transporter region affects serotonin uptake in human blood platelets. Am J Med Genet 88:83–87

Harrison AA, Liem YT, Markou A (2001) Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. Neuropsychopharmacology 25:55–71

Heatherton TF, Kozlowski LT, Frecker RC (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 86:1119–1127

Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP (1996) Allelic variation of human serotonin transporter gene expression. J Neurochem 66:2621–2624

Hitsman B, Pingitore R, Spring B, Mahableshwarkar A, Mizes JS, Seagraves KA, Kristeller JL, Xu W (1999) Antidepressant pharmacotherapy may help some cigarette smokers more than others. J Consult Clin Psychol 67:547–554

Ishikawa H, Ohtsuki T, Ishiguro H, Yamakawa-Kobayashi K, Endo K, Lin Y, Yanagi H, Tsuchiya S, Kawata K, Hamaguchi H, Arinami T (1999) Association between serotonin transporter gene polymorphism and smoking among Japanese males. Cancer Epidemiol Biomarkers Prev 8:831–833

Killen JD, Fortmann SP, Stephen P, Schatzberg AF, Hayward C, Sussman L, Rothman M, Strusberg L, Varady A (2000) Nicotine patch and paroxetine for smoking cessation [article]. J Consult Clin Psychol 68:883–889

Lesch KP, Wolozin BL, Murphy DL, Reiderer P (1993) Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. J Neurochem 60:2319–2322

Linnoila VM, Virkunnen M (1992) Aggression, suicidality, and serotonin. J Clin Psychiatry 53:46–51

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

Macedo T, Ribeiro CA, Cotrim D, Tavares P, Morgadinho MT, Caramona M, Vicente MT, Rodriques L, Cardoso MG, Keating ML (1995) Catecholamine and MHPG plasma levels, platelet levels, platelet MAO activity, and 3H-imipramine binding in heroin and cocaine addicts. Mol Neurobiol 11:21–29

Maguire K, Tuckwell V, Pererira A, Dean B, Singh B (1993) Significant correlation between 14C-5-HT uptake and 3H-paroxetine binding to platelets from healthy volunteers. Biol Psychiatry 34:356–360

Maguire KP, Norman TR, Apostolopoulos M, Judd FK, Burrows GD (1995) Platelet [3H] paroxetine binding in panic disorder. J Affect Disord 33:117–122

Mendez-Alvarez E, Soto-Otero R, Sanchez-Sellero I, Lopez-Rivadulla M, Lamas M (1997) Inhibition of brain monoamine oxidase activity by adducts of 1,2,3,4-tetraishydroisoquinoline with components of cigarette smoke. Life Sci 60:1719–1727

Niaura R, Spring B, Borrelli B, Hedeker D, Goldstein MG, Keuthen N, Depue J, Kristeller J, Ockene J, Prochazka A, Chiles JA, Abrams DB (2002). Multicenter trial of fluoxetine as an adjunct to behavioral smoking cessation treatment. J Consult Clin Psychol 70:887–896

Olausson P, Engel JA, Soderpalm B (2002) Involvement of serotonin in nicotine dependence: processes relevant to positive and negative regulation of drug intake. Pharmacol Biochem Behav 71:757–771

Ozaki N, Rosenthal NE, Mazzola P, Chiveh CC, Hardin T, Garcia-Borreguero D, Schwartz PJ, Turner E, Oren DA, Murphy DL (1994) Platelet [3H]paroxetine binding, 5-HT stimulated Ca2+ response, and 5-HT content in winter seasonal affective disorder. Biol Psychiatry 136:458–466

Patkar AA, Naik P, Mardsen CA, McLean PC, Kendall DA, Al-Chalabi T (1995) Platelet [3H]-binding in type I and type II alcoholism. Hum Psychopharmacol 10:1–6

Patkar AA, Gottheil E, Berrettini WH, Hill KP, Thornton CC, Weinstein SP (2003) Relationship between platelet serotonin uptake sites and measures of impulsivity, aggression and craving among African-American cocaine abusers. Am J Addict (in press)

Pomerleau CS, Pomerleau OF, Majchrzak MJ, Kloska DD, Malakuti R (1990) Relationship between nicotine tolerance questionnaire scores and plasma cotinine. Addict Behav 15:73–80

Rausch JL, Fefferman M, Ladisich-Rogers DG, Menard M (1989) Effect of nicotine on human blood platelet serotonin uptake and efflux. Prog Neuropsychopharmacol Biol Psychiatry 13:907–916

Reuben M, Clarke PB (2000) Nicotine-evoked [3H]5-hydroxytryptamine release from rat striatal synaptosomes. Neuropharmacology 39:290–299

Ribeiro EB, Bettiker RL, Bogdanov M, Wurtman RJ (1993) Effects of systemic nicotine on serotonin release in rat brain. Brain Res 621:311–318

Seth P, Cheeta S, Tucci S, File SE (2002) Nicotinic -serotonergic interactions in brain and behavior. Pharmacol Biochem Behav 71:795–805

Sheline YI, Black KJ, Bardgett ME, Csernansky JG (1005) Platelet binding characteristics distinguish placebo responders from nonresponders. Neuropsychopharmacology 12:315–322

Strachan AT, Maughan RJ (1998) Platelet serotonin transporter density and related parameters in endurance trained and sedentary male subjects. Acta Physiol Scand 163:165–171

Wagenknecht LE, Cutter GR, Haley NJ, Sidney S, Manolio TA, Hughes GH, Jacobs DR (1990) Racial differences in serum cotinine levels among smokers in the Coronary Artery Risk Development in (Young) Adults study. Am J Public Health 80:1053–1056

Xu Z, Seidler FJ, All SF, Slikker W, Slotkin TA (2001) Fetal and adolescent nicotine administration: effects on CNS. Brain Res 914:166–178