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Association of Long-Term Nicotine Abstinence With Normal Metabotropic Glutamate Receptor-5 Binding

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ABSTRACT

BACKGROUND: Nicotine addiction is a major public health problem and is associated with primary glutamatergic dysfunction. We recently showed marked global reductions in metabotropic glutamate receptor type 5 (mGluR5) binding in smokers and recent ex-smokers (average abstinence duration of 25 weeks). The goal of this study was to examine the role of mGluR5 downregulation in nicotine addiction by investigating a group of long-term ex-smokers (abstinence >1.5 years), and to explore associations between mGluR5 binding and relapse in recent ex-smokers.

METHODS: Images of mGluR5 receptor binding were acquired in 14 long-term ex-smokers, using positron emission tomography with radiolabeled [11C]ABP688, which binds to an allosteric site with high specificity.

RESULTS: Long-term ex-smokers and individuals who had never smoked showed no differences in mGluR5 binding in any of the brain regions examined. Long-term ex-smokers showed significantly higher mGluR5 binding than recent ex-smokers, most prominently in the frontal cortex (42%) and thalamus (57%).

CONCLUSIONS: Our findings suggest that downregulation of mGluR5 is a pathogenetic mechanism underlying nicotine dependence and the high relapse rate in individuals previously exposed to nicotine. Therefore, mGluR5 receptor binding appears to be an effective biomarker in smoking and a promising target for the discovery of novel medication for nicotine dependence and other substance-related disorders.

Keywords: Abstinence, Addiction, Glutamate, mGluR5, Nicotine, Relapse

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The recidivism rate for nicotine consumption is extraordinarily high. Each year, 40% of smokers try to quit but only 3% to 5% achieve prolonged abstinence for 6 to 12 months after a given quit attempt (1). Although the neurobiological mechanisms underlying relapse are largely unknown, there is increasing evidence that molecular and neurochemical adaptations in the glutamatergic system play an important role in the recidivism for cocaine and nicotine abuse (2).

There is strong evidence that the metabotropic glutamate receptor type 5 (mGluR5) has a specific role in addiction. In 2001, Chiamulera *et al*. (3) demonstrated that mGluR5 gene knock-out mice do not respond to acute administration of various doses of cocaine and fail to acquire intravenous self-administration of cocaine. Multiple studies have demonstrated that negative allosteric modulators of the mGluR5 receptor, such as 2-methyl-6-(phenylethynyl)pyridine (MPEP) and 3-(2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP), reduce the self-administration of addictive drugs such as cocaine and nicotine (4–10). In addition, there is direct evidence that mGluR5 receptor antagonism attenuates reinstatement to nicotine (6,7).

We used positron emission tomography (PET) to measure mGluR5 availability by using the radiolabeled mGluR5 antagonist 3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-*O*-^{11}C-methyl-oxime ([^{11}C]ABP688) (11), which binds with high selectivity to an allosteric site. We previously showed a marked reduction in mGluR5 at that time, measured as distribution volume ratio (DVR) (total distribution volume/volume of non-displaceable radioligand) based on the cortical uptake-to-cerebellum uptake ratio at equilibrium in a bolus infusion setting (12). We found a strong reduction in gray matter DVR between smokers and nonsmokers (20.6% and between ex-smokers and nonsmokers (11.5%). Another research group (13) has recently replicated our findings in smokers.

To test the association between relapse of nicotine addiction and mGluR5 binding, we conducted two follow-up studies. First, we measured mGluR5 binding in subjects with a long duration of abstinence (78 to 1144 weeks). Given that these subjects belong to approximately 3% of ex-smokers with the ability to abstain for a longer period (1), we expected their mGluR5 binding to be positively associated with abstinence duration. Second, we followed the ex-smokers assessed in our previous study over time and tested for an association between relapse and mGluR5 binding in that subsample. Based on our previous study, we hypothesized that abnormally low mGluR5 binding predicts a high risk of relapse. In this paper, we have changed our nomenclature to that in the study by Innis *et al*.

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(14). Upon request, we did not calculate DVR but binding potential of nondisplaceable radioligand (BP_{ND}), which is calculated as [$V_T/V_{ND} - 1$], and therefore, we had to recalculate the outcome variables, which resulted in percent changes that were different from those in our previous publication, although the raw uptake data remained the same.

METHODS AND MATERIALS

Subjects

Participants were recruited by using local newspaper advertisements and were screened at Zurich University hospital. Inclusion criterion for the group of long-term ex-smokers was duration of nicotine abstinence of more than 1.5 years, having smoked at least 11 cigarettes per day.

Exclusion criteria included neurological or medical disorders, pregnancy, breast-feeding, history of psychosis, manic episodes, current depressive episodes, substance dependence, and autism (based on clinical interview as described below). Subjects were enrolled in the study after a full explanation of the study design and procedures and after written consent was obtained, which was approved by the local ethics committee (Kantonale Ethikkommission Zürich).

All subjects were assessed using an unstructured clinical interview by a psychiatrist and the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision. Clinical measures included the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI). Magnetic resonance images were assessed for each subject to exclude any structural brain pathology.

At the time of the PET scan, an additional clinical evaluation consisting of a physical examination and completion of the BAI and BDI was performed. Scores from the BAI and BDI were used for further statistical evaluations.

Subjects in the recent ex-smoker group were questioned by telephone regarding potential relapse during the preceding 5 to 12 months (mean, 8.4 ± 2.5 months) after the scanning session. They were asked if and when they had started smoking again and their daily cigarette consumption.

Positron Emission Tomography

The description of PET with [^{11}C]ABP688 can be found in detail in a previous study (12). In this article, we changed the outcome variable from previously defined DVR to BP_{ND} at equilibrium (14). In the current study we included a new group of long ex-smokers, with whom we used the same bolus/infusion protocol as with the previous study with [^{11}C]ABP688 in smokers (12). We showed previously that the bolus infusion method gave reliable results using the cerebellum as reference region (15,16).

Statistical Analysis

We used PMOD version 3.0 software (PMOD Technologies, Zurich, Switzerland) for all analyses. Uptake images were transformed to a common space, the Montreal Neurological Institute template. The same 23 region of interest (ROI) definitions used in our previous study were used in the present study (except for brain stem). These ROI included 2 regions within the cingulate gyrus (anterior and posterior), 4 cortical regions (frontal, parietal, temporal, and occipital), 3 regions in the limbic system (medial orbitofrontal cortex, amygdala, and medial temporal lobe), and 3 regions in the prosencephalon (caudate, putamen, and thalamus) (12). The same gray matter mask created for the previous study was used in this analysis. To test for differences in mGluR5 BP_{ND} between the groups, we used two-sample two-tailed *t*-tests. Spearman correlations were used to assess the relationship between clinical variables and mGluR5 BP_{ND}.

RESULTS

The clinical characteristics of 14 smokers, 14 age- and gender-matched nonsmokers, 14 recent ex-smokers, and 14 long-term ex-smokers are shown in Table 1. Only 1 to 2 subjects per group had a history of 1 episode of depression but did not show any signs of depression at the time of scanning. Age did not differ across the groups ($F_{3,55} = 2.46$, $p > .05$), and there were no significant differences in age between male and female subjects overall or within the smoker, nonsmoker, recent ex-smoker, or long-term ex-smoker groups ($F_{1,55} = .03$, $p > .05$). The BDI scores across all groups did not differ ($F_{3,55} = .55$, $p > .6$). BAI scores on the day of scanning were significantly different across all groups ($F_{3,55} = 3.2$, $p < .05$). Scheffe's post hoc test results revealed that this significant effect was not due to the comparisons with long-term ex-smokers ($p > .4$ in all cases). There were no significant differences in the numbers of cigarettes smoked per day ($t_{26} = .05$; $p > .9$), the number of years smoking ($t_{26} = .7$; $p > .4$), or the age of onset ($t_{26} = .02$; $p > .9$) between recent and long-term ex-smokers. There were no significant difference in number of cigarettes smoked per day ($t_{26} = .9$; $p > .3$), number of years smoking ($t_{26} = .66$; $p > .5$), or age of onset ($t_{26} = .96$; $p > .3$) between long-term ex-smokers and smokers.

We did not see a significant effect of age of smoking onset on mGluR5 BP_{ND} in the long-term ex-smoker group, after correction for multiple comparisons. Testing for the effect of age of smoking onset as a covariate in a repeated measures analysis of all smoking groups, we did not see a significant difference between effects of this factor ($F_{1,37} = .026$, $p > .8$), nor did we see a potential effect of the variable age in this comparison among all groups ($F_{1,50} = 3.27$, $p > .05$).

We also performed a repeated measures analysis (regions) combining all groups and did not find a significant difference between subjects for effect of gender on the mGluR5 binding ($F_{1,53} = .225$, $p > .6$). In the long-term ex-smoker group, there were no differences in mGluR5 BP_{ND} between male and female subjects ($p > .05$ for all ROIs), with the exception of the left mediotemporal region, which showed an uncorrected significant difference in mGluR5 BP_{ND} ($p < .05$). The mean [^{11}C]ABP688 activity did not differ significantly among nonsmokers, smokers, recent ex-smokers, and long-term ex-smokers (729 ± 71, 765 ± 80, 740 ± 34, and 697 ± 61 MBq, respectively). Table 2 and Figure 1 summarize the results of the mGluR5 BP_{ND} comparisons across the four clinical groups in the 23 ROIs examined. Overall, there was a significant region × group effect ($F_{66,99} = 2.214$; $p < .0001$). The post hoc comparisons between long-term ex-smokers and all other groups revealed that all regions showed a