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Baseline expression of $\alpha 4 \beta 2^*$ nicotinic acetylcholine receptors predicts motivation to self-administer nicotine

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Abstract

Background—Marked inter-individual differences in vulnerability to nicotine dependence exist, but factors underlying such differences are not well understood. The midbrain $\alpha 4 \beta 2^*$ subtype of nicotinic acetylcholine receptors (nAChRs) has been implicated in mediation of the reinforcing effects of nicotine responsible for dependence. However, no study has been performed evaluating the impact of inter-individual differences in midbrain nAChR levels on motivation to self-administer nicotine.

Methods—Baseline levels of $\alpha 4 \beta 2^*$ nAChRs were measured using 2-[¹⁸F]fluoro-A-85380 (2-FA) and positron emission tomography (PET) in five squirrel monkeys. Motivation to self-administer nicotine was subsequently measured using a progressive-ratio (PR) schedule of reinforcement.

Results—Greater motivation to self-administer nicotine was associated with lower levels of midbrain nAChRs.

Conclusions—The results suggest that level of expression of nAChRs is a contributing factor in the development of nicotine dependence. Similarly, it has been previously shown that low levels of dopamine D₂ receptors (DRD2) are associated with a higher preference for psychostimulant use in humans and non-human primates. Together, results from these PET studies of dopaminergic and nicotinic cholinergic transmission suggest that an inverse relationship between the availability of receptors that mediate reinforcement and the motivation to take drugs exists across different neurotransmitter systems.

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Keywords

positron emission tomography; non-human primates; nicotinic receptors; nicotine self-administration; *in vivo* binding

INTRODUCTION

The $\alpha 4\beta 2^*$ subtype of nicotinic acetylcholine receptors (nAChRs), located in the midbrain area, has been implicated in mediating the reinforcing effects of nicotine (1). Animal models using genetically modified rodents implicate the involvement of these receptors in the development of nicotine dependence, since $\beta 2$ subunit deletion decreases sensitivity to nicotine's reinforcing effects and $\alpha 4$ subunit modifications that increase sensitivity to nicotine result in increased sensitivity to nicotine reinforcement (2,3). Together, these results suggest the hypothesis that a high baseline level of midbrain nAChRs will be associated with a high motivation to self-administer nicotine. No studies have yet been performed to evaluate this hypothesis. Since those studies are not ethical in humans, to test this hypothesis we used adult male squirrel monkeys that had learned to self-administer nicotine (4,5). To determine the baseline level of midbrain nAChRs we measure a binding potential (BP_{ND}) of 2-[^{18}F]fluoro-A-85380 (2-FA), a selective $\alpha 4\beta 2^*$ nAChR PET ligand. BP_{ND} is proportional to the density of receptors available for radioligand binding *in vivo*.

MATERIAL AND METHODS

Subjects

Five adult drug-naïve male squirrel monkeys (*Saimiri sciureus*), weighing 730 to 950 g, were housed individually in a temperature- and humidity-controlled room and were maintained on a 12-h light/dark cycle; the lights were on from 6:45 AM to 6:45 PM. Experiments were conducted during the light phase. Monkeys were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animals (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, and the Guide for Care and Use of Laboratory Animals (National Research Council 2003).

PET imaging studies

Radiochemistry: [^{18}F]Fluoride was produced using an RDS111 negative ion cyclotron, and 2-FA was synthesized using a modified semiautomated method (6). The final product was formulated as a sterile and pyrogen-free isotonic solution. Radiochemical purity product was greater than 98% and specific activity was in the range from 275 to 516 GBq/ μ mol (450 ± 130 GBq/ μ mol, average $\pm$ SD).

PET and MRI scanning procedures

Data were acquired on a Siemens Exact ECAT HR+ tomograph (63 slices, center to center spacing of 2.4 mm, with an in-plane reconstructed resolution, full width at half maximum (FWHM), of 4.7 mm at the center of the field of view and reconstructed axial spatial resolution of 4.2 mm in 3D mode). Before each radioligand administration, transmission scans were obtained with three rotating ^{68}Ge - ^{68}Ga sources and to be used to correct for photon attenuation by tissues and mask. PET images were reconstructed from the raw data with a standard filtered-back projection algorithm and a RAMP filter. For the PET scans, monkeys were initially anesthetized with 1.5 mg/kg alfadolone and alfaxolone acetate (Saffan[®], Arnolds Veterinary Products, Shropshire, U.K.), given intramuscularly. Anesthesia was then maintained by 1.5–

2.5% isoflurane. An individually molded thermoplastic face mask was secured to a custom-made monkey head-holder attached to a backboard.

Acquisition of dynamic PET scans ($n=5$) started with the injection of 2-FA as a bolus (47 ± 15 MBq/kg injected intravenously in approximately 1 ml of saline over 20 sec) and continued for 5 hours.

Anatomical MRI brain images were acquired on a 3.0 Tesla Siemens Magnetom Allegra MRI unit (Siemens Medical Solutions) using continuous intravenous infusion of 8–11 mg/kg/h Saffan to maintain anesthesia.

Vital signs, including heart rate, ECG (during PET studies), respiration rate, ET_{CO_2} and blood oxygen saturation (always maintained above 95%), were continuously monitored during the studies.

PET data analysis

Regions of interest (ROIs) for thalamus, midbrain and cerebellum were drawn on the individual T1 MRI images co-registered to PET images, with reference to a stereotaxic atlas. ROIs for the muscles were placed on the back of the neck, in the area of the semispinalis cervicis, splenius capitis, and obliquus capitis muscles. BP_{ND} values were calculated using a four-parameter reference tissue model (PMOD v. 2.75). For receptor quantification using muscles as a reference region, BP_{ND} values were corrected for differences between brain tissue V_{ND} and muscle V_T using equation (6) from (7).

BP_{ND} parametric images were generated using PMOD v. 2.75 (Gunn method) and cerebellum as the reference region. After co-registration of BP_{ND} parametric maps to individual MRI images used to obtain the average MRI image, BP_{ND} parametric maps were superimposed on the average MRI image without spatial normalization.

Intravenous nicotine self-administration

Several days after obtaining PET data, acquisition sessions were initiated during which the monkeys were allowed to self-administer nicotine intravenously under a fixed-ratio schedule of reinforcement. Subsequently, they were switched to a progressive-ratio schedule of reinforcement (see (5) and supplemental material for details). Since there was an inverted U-shaped dose-effect curve under this schedule, we focused on responding maintained by a 30 μ g/kg per injection dose of nicotine. This dose produces peak levels of injections per session, peak breaking point values and a near maximal nicotine intake per session (5).

RESULTS

Contrary to our initial hypothesis, animals with low baseline levels of midbrain $\alpha 4\beta 2^*$ nAChRs (low BP_{ND}) exhibited a higher motivation to self-administer nicotine, as assessed by a number of responses made under the progressive-ratio schedule (Fig. 1a and Fig. 1c). There was a significant negative correlation between the number of responses and midbrain BP_{ND} values calculated using either cerebellum or muscle as reference regions (rank correlations for both, $\rho = -1$, $P < 0.05$). The fact that similar results were obtained using either cerebellum or muscle as reference region suggests that any confounding role of nicotinic receptor expression within the cerebellum in these measurements was likely inconsequential. We should mention that it was not possible to analyze BP_{ND} values in other brain areas that may be involved in reinforcement, such as striatum, because of the low receptor density and therefore unreliable specific binding signal obtained with 2-FA in those areas (7). The thalamus is a brain area which displays high levels of $\alpha 4\beta 2^*$ nAChRs that can be measured by 2-FA. We found no significant correlation between the number of responses and thalamus BP_{ND} values (Fig. S1).

This may reflect the lack of involvement of the thalamus in nicotine reinforcement processes. A trend was noted toward an inverse correlation between thalamus BP_{ND} values and number of responses under the PR schedule (Fig. S1), which might be due to a correlation between the levels of $\alpha 4\beta 2^*$ nAChRs in the two structures (Fig. S2), but this correlation did not reach significance.

Data obtained during the acquisition phase under the FR schedule of reinforcement were also analyzed, since responses during acquisition may also reflect motivational effects of nicotine. There was a significant negative correlation between the number of days needed to reach the final FR-10 response requirement and the number of nicotine injections self administered per session under the final FR-10 schedule ($P < 0.05$). However, this result should be viewed with caution, due to the limited number of animals and the absence of strict criteria for increasing ratio requirements during the acquisition phase. We found no significant correlation between BP_{ND} values calculated using either cerebellum or muscle as reference regions and the number of nicotine injections under the FR-10 schedule (all $P > 0.23$). This may reflect either a different motivational aspects of nicotine-taking assessed by FR and PR schedules or a limitation of one-hour access to nicotine under the FR schedule to detect such correlation.

The observation that a low level of baseline midbrain nAChRs was associated with a high motivation to self-administer nicotine is consistent with previous findings obtained with dopamine receptors and psychostimulant addiction. For example, low levels of DRD₂ expression are associated with a higher preference for psychostimulant use in humans (8) and a greater motivation to self-administer cocaine in monkeys (9). Furthermore, similar to blockade of DRD₂ reducing the reinforcing effects of psychostimulant drugs (10), blockade of nAChRs reduces the reinforcing effects of nicotine (11,12). It should be noted that in the above referenced DRD₂ PET studies, as well as in the present study, BP_{ND} was used as an *in vivo* measure of receptor density. Since BP_{ND} is directly proportional to the density of receptors available for radioligand binding (receptors not occupied by an endogenous transmitter), the observed inter-subject variations in BP_{ND} for DRD₂ and nAChRs may reflect potential differences in levels of endogenous dopamine and acetylcholine, respectively. Taken together, those studies of dopaminergic and nicotinic cholinergic transmission suggest that an inverse relationship between the availability of receptors that mediate reinforcement and the motivation to take drugs exists across different neurotransmitter systems.

CONCLUSION

Although these results rely on a limited sample size and should be duplicated with a larger group of animals, this report is the first to show that baseline level of nAChRs may affect the motivation to self-administer nicotine. A low level of $\alpha 4\beta 2^*$ nAChRs could be one of the predisposing factors for nicotine dependence and measuring levels of nAChRs or exploring genetic factors that control baseline levels of $\alpha 4\beta 2^*$ nAChRs may allow an assessment of individual risk of developing tobacco dependence, which remains the leading preventable cause of death in developed countries.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

a. $\alpha 4\beta 2$ * nAChRs (BP_{ND} of 2-FA) at the level of the midbrain. Upper left image – an average T1 brain MRI from all five squirrel monkeys; #1 – #5 BP_{ND} images from each individual monkey superimposed on the average MRI and sorted by the number of responses during the progressive-ratio schedule of reinforcement. Note that, only voxels with BP_{ND} values > 1 are shown for clarity. **b.** Typical cumulative-response records of responding on the active lever under the progressive-ratio schedule of reinforcement maintained by 30 $\mu\text{g/kg}$ /injection dose of nicotine. Representative records are shown for a monkey with ‘high’ (#5) and a monkey with ‘low’ (#1) motivation to self-administer nicotine. **c.** Inverse correlation between BP_{ND} values measured in the midbrain (correlation shown here was obtained using cerebellum as a

reference region) and number of responses made by the monkeys on the active lever during the progressive-ratio schedule of intravenous nicotine self-administration.