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Decreased vesicular monoamine transporter, type 2 availability in the striatum following chronic cocaine self-administration in nonhuman primates

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Abstract

BACKGROUND—Consistent with postmortem data, in a recent positron emission tomography (PET) study, we demonstrated less [¹¹C]-(+)-α-dihydrotetrabenazine (DTBZ) binding to striatal vesicular monoamine transporter, type 2 (VMAT2) in cocaine abusers compared to controls. A major limitation of these between-group comparison human studies is their inability to establish a causal relationship between cocaine abuse and lower VMAT2. Furthermore, studies in rodents that evaluated VMAT2 binding before and after cocaine selfadministration do not support a reduction in VMAT2.

METHODS—To clarify these discrepant VMAT2 findings and attribute VMAT2 reduction to cocaine abuse, we imaged four rhesus monkeys with [¹¹C]DTBZ PET before and after 16-months of cocaine self-administration. [¹¹C]DTBZ binding potential (BP_{ND}) in the striatum was derived using the simplified reference tissue method with the occipital cortex time activity curve as an input function.

RESULTS—Chronic cocaine self-administration led to a significant (25.8 ± 7.8%) reduction in [¹¹C]DTBZ BP_{ND}.

CONCLUSIONS—In contrast to the cocaine rodent investigations that do not support alterations in VMAT2, these results in nonhuman primates clearly demonstrate a reduction in VMAT2 binding following prolonged exposure to cocaine. Lower VMAT2 implies that fewer dopamine storage vesicles are available in the pre-synaptic terminals for release, a likely factor contributing to decreased dopamine transmission in cocaine dependence. Future studies should attempt to

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DISCLOSURES

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clarify the clinical significance of lower VMAT2 in cocaine abusers, for example, its relationship to relapse and vulnerability to mood disorders.

Keywords

[¹¹C]-(+)-dihydrotetrabenazine (DTBZ); Positron emission tomography; and vesicular monoamine transporter; type 2 (VMAT2); cocaine; self-administration; nonhuman primates

INTRODUCTION

Positron emission tomography imaging studies have demonstrated decreased amphetamine-induced dopamine release in the striatum of chronic cocaine abusers relative to matched healthy controls (1, 2). These initial reports have been followed by data suggesting that less dopamine release is associated with higher relapse rates in cocaine abusers (3, 4). The pre- and post-synaptic mechanisms that lead to blunted dopamine release in cocaine abusers remain unknown. One possible mechanism for lower amphetamine-induced dopamine release is that fewer dopamine storage vesicles are available in the pre-synaptic terminals for release. Consistent with this premise, postmortem studies in cocaine abusers have reported a reduction in striatal vesicular monoamine transporter, type 2, the membrane protein that regulates the size of the vesicular dopamine pool, relative to healthy subjects (5–8). In the first *in vivo* PET investigation of VMAT2 in recently abstinent cocaine abusers, we corroborated the postmortem finding of lower striatal VMAT2 relative to matched controls using the VMAT2 specific PET radioligand [¹¹C]DTBZ (9). The 10 to 16% reduction in VMAT2 in the striatal subdivisions in cocaine abusers shown in this study indicates a compensatory down-regulation of the pre-synaptic dopamine storage vesicles, a loss of dopaminergic terminals, or a combination of both. A major limitation of these postmortem and imaging studies that contrasted groups of cocaine abusers with controls is their inability to establish a causal link between cocaine abuse and lower VMAT2. This issue is further complicated by the fact that no such lowering in VMAT2 binding is evident in rodents studied before and after exposure to chronic cocaine in a within-subject design (10–13). There are risks in the exclusive reliance on rodents as a model for cocaine addiction in humans because the published basic science and clinical imaging literature in this area are quite discordant (for detailed review, refer to 14). Thus, to clarify the discrepant human and rodent VMAT2 findings, and attribute VMAT2 alterations to chronic cocaine abuse we evaluated the *in vivo* status of VMAT2 before and after chronic cocaine self-administration in nonhuman primates.

MATERIALS AND METHODS

General Design

All experiments were performed under a protocol that was reviewed and approved by the University of Pittsburgh Institutional Animal Care Use Committee. A total of 12 [¹¹C]DTBZ PET scans in 8 adult male rhesus monkeys acquired over the course of 20 months are reported here.

1. Cocaine self-administration and [^{11}C]DTBZ BP_{ND}—The aim of these within-subject experiments was to demonstrate that chronic cocaine self-administration lowers [^{11}C]DTBZ BP_{ND}. Four animals were scanned with [^{11}C]DTBZ and PET, once at baseline and again following ~16-months of cocaine self-administration. All four animals that participated in this aim were abstinent from cocaine for ~22 months prior to the baseline PET scan. Cocaine self-administration methods were consistent with previously published methods (15). Typical self-administration schedule involved 6–8 infusions of cocaine (0.5–0.6 mg kg⁻¹ per infusion under an fixed-ratio 30 schedule with 10 min timeout between infusions) four days a week. Prior history and current experimental exposure to cocaine in the animals is detailed in Table 1. To minimize the acute effects of cocaine on endogenous dopamine and VMAT2 binding (16–19), the cocaine-self administering animals were scanned three weeks following their last use of cocaine. This also ensured consistency with our previous [^{11}C]DTBZ imaging study in which cocaine abusing humans were studied following a minimum of two weeks of monitored abstinence (9). The baseline [^{11}C]DTBZ BP_{ND} was then contrasted with the post-cocaine [^{11}C]DTBZ BP_{ND} acquired in the same animals roughly 16 months later.

2. Age and [^{11}C]DTBZ BP_{ND}—Prior imaging in healthy humans and nonhuman primates has shown that [^{11}C]DTBZ BP_{ND} declines by a relatively modest 0.5% to 1.5% per year (20, 21). In order to demonstrate that the contribution of aging (~16-month duration) to [^{11}C]DTBZ BP_{ND} in the animals that self-administered cocaine animals was small, we scanned four control animals that were closely matched in age and daily activities with the cocaine animals, which had no prior exposure to cocaine. The [^{11}C]DTBZ BP_{ND} values in these control animals was then contrasted with the baseline [^{11}C]DTBZ BP_{ND} values (data from the experiments listed in section above) in cocaine animals that were acquired ~20 months ago. This contrast provided an estimate of how much aging contributed to the reduction in [^{11}C]DTBZ BP_{ND}.

Imaging methods

Prior to PET Imaging, each animal underwent a structural MRI scan using a Siemens 3T Allegra scanner for localization of regions of interest (22). [^{11}C]DTBZ was synthesized using the methodology reported previously by Kilbourn, et al. (23). Fasted animals were immobilized with ketamine (10 mg kg⁻¹ intramuscularly, approximately 90 minutes prior to the injection of radiotracer) and anesthetized with 1 to 2% isoflurane via endotracheal tube. The animals' vital signs were monitored every 10 min and temperature was kept constant at 37°C with heated water blankets. An intravenous perfusion line was placed in each animal for hydration and injection of the radiotracer. PET imaging was performed using a Siemens microPET P4 scanner, which has a spatial resolution of ~2 mm full width half-maximum (FWHM) (24). A 10-minute transmission scan was obtained prior to radiotracer injection for attenuation correction. Activity was injected intravenously over 30 seconds as a bolus. Emission data were collected for 60 min post-injection in 20 acquisition frames ranging in duration from 30 seconds to 10 minutes.

PET data were reconstructed using filtered back-projection and corrected for photon attenuation, dead time, scatter, and radioactive decay. Reconstructed image files were then

processed with the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm). MR-PET image alignment was performed using a mutual information algorithm implemented in SPM2. Regions of interest including the striatum and occipital cortex (reference region) were delineated on the structural magnetic resonance image (MRI) and then transferred to the co-registered PET scan. Time activity curves were generated for these regions using the methods outlined previously (25). Activity from the right and left regions of interest was averaged.

The outcome measure BP_{ND} ($= f_{ND} * B_{avail}/K_D$, where B_{avail} is the density of VMAT2 available to bind the radioligand *in vivo*, K_D is the disassociation constant and f_{ND} is the free fraction in the nondisplaceable compartment) was derived for [^{11}C]DTBZ using the simplified reference tissue model with the occipital cortex time activity curve as an input function (26). The relative change in [^{11}C]DTBZ BP_{ND} (BP_{ND}) in the cocaine self-administration experiments were calculated as the difference between BP_{ND} measured after cocaine exposure ($BP_{ND\ COC}$) and BP_{ND} measured at baseline ($BP_{ND\ BASE}$), and expressed as a percentage of $BP_{ND\ BASE}$.

$$\Delta BP_{ND} = 100\% * \frac{BP_{ND\ COC} - BP_{ND\ BASE}}{BP_{ND\ BASE}}$$

Statistical analysis

All statistical analysis was performed with IBM SPSS Statistics 20. The differences in scan variables (such as injected mass and dose) and outcome measures between conditions (cocaine and age) were contrasted using either paired (within-animal comparisons) or unpaired t tests (between-animal). A two-tailed $p = 0.05$ was selected as the significance level for all tests. All values are expressed as mean $\pm$ standard deviation unless specified.

RESULTS

1. Cocaine self-administration and [^{11}C]DTBZ BP_{ND}

Demographic variables—The mean age of the animals at the time of the baseline and post-cocaine [^{11}C]DTBZ PET scans was 99 ± 5 and 115 ± 6 months. The mean weight of the animals at the time of the baseline and post-cocaine evaluation was 9.7 ± 1.1 kg and 9.9 ± 0.1 kg ($t = 0.38$, $df = 3$, $p = 0.73$).

Injected dose and mass—The mean injected dose for [^{11}C]DTBZ was 8.7 ± 1.6 mCi and 8.9 ± 1.7 mCi in the baseline and post-cocaine conditions ($n = 4/\text{condition}$; $t = 1.79$, $df = 3$, $p = 0.17$). The mean specific activity at the time of injection for [^{11}C]DTBZ was 4448 ± 1666 Ci mmol^{-1} and 3005 ± 755 Ci mmol^{-1} in the baseline and post-cocaine conditions ($t = -2.86$, $df = 3$, $p = 0.07$). The mean injected mass for [^{11}C]DTBZ was 0.68 ± 0.21 μg and 0.96 ± 0.12 μg in the baseline and post-cocaine conditions ($t = 2.55$, $df = 3$, $p = 0.08$).

Binding potential—Chronic cocaine self-administration led to a significant reduction in [^{11}C]DTBZ BP_{ND} in the striatum ($BP_{ND} = 25.8 \pm 7.8\%$; $t = -6.32$, $df = 3$, $p = 0.008$, see Figure 1).

2. Age and [^{11}C]DTBZ BP_{ND}

Demographic variables—The mean age of the control animals at the time of the [^{11}C]DTBZ PET scans was 119 ± 6 months. The mean weight of the control animals at the time of the scan was 10.4 ± 1.7 kg.

Injected dose and mass—The mean injected dose, specific activity and injected mass for [^{11}C]DTBZ was 9.1 ± 1.5 mCi, 3472 ± 1325 Ci mmol⁻¹ 0.93 ± 0.30 μg . There were no significant difference between these scan parameters and that in cocaine self-administering animals in the baseline condition (in previous section, all p values > 0.2)

Binding potential—The contribution of aging was not significant when the BP_{ND} in age-matched control animals (3.65 ± 0.54) was contrasted with the baseline BP_{ND} values measured in cocaine animals ~20 months ago (3.80 ± 0.57 ; unpaired t test, $t=0.382$, $df=6$, $p=0.72$; see Figure 2). The contrast of BP_{ND} in age-matched controls with post-cocaine self-administration BP_{ND} was at trend level (unpaired t test, $t= -2.291$, $df=6$, $p=0.06$, see Figure 2).

DISCUSSION

The primary finding of this nonhuman primate imaging study is that chronic cocaine self-administration leads to a profound (25%) reduction in striatal VMAT2 availability. These results are consistent with the human postmortem (12–22%) and imaging (10–16%) that have reported lower VMAT2 in cocaine abusers compared to controls (5–9). The animals in this study self-administered an intravenous dose of ~ 43 mg kg⁻¹ per month for 16- months (cumulative cocaine dose of 694 mg/16 months). Crack-cocaine abusing humans in previous PET investigations report using a minimum of 16 grams of cocaine per month (1). Assuming a body weight of 75 to 100 kilograms and a bioavailability of 45% for smoked crack-cocaine in humans this corresponds to 72 to 96 mg kg⁻¹ per month (27). This would suggest that the dose of chronic cocaine administered by animals in this study is 50% less than that abused by cocaine-addicted humans. Nevertheless, because the amount of cocaine abused by humans relies on the addicts' ability to provide a reliable history and is subject to factors such as variable purity, bioavailability etc., we need to interpret this comparison with caution.

The strengths of this study include the use of a within-subject design in which [^{11}C]DTBZ BP_{ND} was compared in the same animals before and after cocaine self-administration; prospective documentation of the amount of cocaine self-administered by the animals; and a cocaine-free abstinence period of two weeks prior to the scans. The primary limitation of this study was lack of use a within-subject design to study control animals. This would have provided us with information on the stability (i.e., test-retest variability) of [^{11}C]DTBZ BP_{ND} over the sixteen-month cocaine self-administration period. Thus, to quantify the age-induced reduction in [^{11}C]DTBZ BP_{ND} as a contributor to the BP_{ND} measured following chronic cocaine, we scanned four age-matched control animals that resided in the same colony. The results in these age-matched controls were suggestive of a relatively small 3.9% decrease in [^{11}C]DTBZ BP_{ND} over 20 months ($3.65/3.80$, from BP_{ND} values in Figure 2). This result is inline with a previous nonhuman primate study that suggests $\sim 1.5\%$ decrease

in [^{11}C]DTBZ BP_{ND} over a period of 12 months (21). Also, consistent with this is a report in humans that suggests the age-induced decline in [^{11}C]DTBZ BP_{ND} is a relatively small 0.5% per year (20). Based on these data, it is possible to attribute the relatively large decrease in [^{11}C]DTBZ BP_{ND} in cocaine animals to a chronic cocaine effect. Other methodological limitations include the lack of measurement of chronic cocaine-induced changes in [^{11}C]DTBZ nonspecific binding (V_{ND}), plasma free fraction (f_p), striatal volume, and blood flow in these animals—all of which in theory could impact the measurement of [^{11}C]DTBZ BP_{ND}. The fact that we found no between-group differences in V_{ND} and striatal volume between cocaine abusers and controls in a previous human study somewhat alleviates the concern that these two parameters may have influenced the results. Future studies should confirm the impact of these imaging parameters on cocaine-induced decrease in [^{11}C]DTBZ BP_{ND}. Finally, the fact that amphetamine-induced dopamine release was not measured in the same chronic cocaine animals limits the translational significance of directly linking less VMAT2 to blunted dopamine transmission in addiction. Despite the limitations, the results of this nonhuman primate study for the first time unequivocally establish a causal link between lower VMAT2 and chronic cocaine abuse.

The decrease in VMAT2 binding following chronic cocaine in this nonhuman primate study was not predicted by prior rodent investigations, which are consistent in reporting either no change or an increase (10–13). This discrepancy in VMAT2 binding between the chronic cocaine human and rodent data may very well explain the paradoxical findings observed with regards to acute amphetamine challenge across species. In human cocaine abusers, a blunting of dopamine release is observed, whereas rodents repeatedly exposed to cocaine demonstrate a dopamine sensitization response (or increased dopamine release) following an acute amphetamine challenge. The lower VMAT2 data raise an interesting possibility that perhaps chronic and repeated exposure to cocaine represent a loss or death of dopaminergic nerve terminals in humans, but not in rodents, which are typically exposed to cocaine for a relatively shorter duration of time (weeks in rodents compared to years in humans) in the laboratory. Such toxicity to dopamine neurons is likely to lead to the profound reduction in stimulant induced dopamine release in cocaine dependence. Consistent with such an irreversible neurotoxic effect of cocaine, a recent postmortem investigation showed fewer dopamine cells and increased microglial activation in cocaine abusers compared to controls (28). Further replication of this finding using *in vitro* and *in vivo* methods are necessary to confirm chronic cocaine-induced neurotoxicity in human addicts. Alternatively, lower VMAT2 in chronic cocaine abusers may represent a reversible compensatory down-regulation of vesicular dopamine stores in response to repeated cocaine-induced dopamine release. Future investigations should evaluate whether the low VMAT2 in chronic cocaine abusing humans and/or nonhuman primates measured in early abstinence is reversible with more prolonged periods of abstinence.

In summary, we replicated our initial finding of lower VMAT2 availability in human cocaine abusers in a nonhuman primate model of cocaine dependence. The use of a within-subject design in nonhuman primates for the first time unambiguously establishes a causal link between chronic cocaine abuse and lower VMAT2 binding. However, these results do not exclude the possibility that lower VMAT2 binding could predate drug use in human

cocaine abusers. Alterations in vesicular monoamine transporter availability may be one of the several mechanisms that contribute to decreased monoamine transmission in cocaine dependence. The clinical implications of this may explain the relatively high rates of relapse to cocaine and vulnerability to mood disorders in cocaine abusers. Further research is warranted to understand these clinical issues in cocaine dependence.

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Figure 1. $[^{11}\text{C}]\text{DTBZ}$ BP_{ND} measured at baseline and following chronic cocaine self-administration. Chronic cocaine self-administration led to a significant reduction in $[^{11}\text{C}]\text{DTBZ}$ BP_{ND} . The BP_{ND} in the four animals studied were -26% , -24% , -36% and -17% .

Figure 2. shows no significant difference between [¹¹C]DTBZ BP_{ND} measured in age matched control animals (shaded bar) and pre-cocaine baseline measured approximately 20 months ago (white bar). Also, consistent with our previous study in human cocaine abusers the [¹¹C]DTBZ BP_{ND} in cocaine self-administering animals (black bars) trends lower compared to age matched controls (shaded bars). Error bars shown represent standard deviation

Table 1

Exposure history of animals that self-administered cocaine

Animal	Past cocaine exposure			Washout period	Current cocaine exposure		
	Age (months)	Duration (months)	Cumulative dose (mg kg ⁻¹)		Age (months)	Duration (months)	Cumulative dose (mg kg ⁻¹)
A	67	12	540	22	100	16	676.0
B	58	11	503	21	90	16	707.1
C	68	12	524	22	102	16	681.9
D	68	12	516	22	102	16	711.3
<hr/>							
Mean + standard deviation	65 ± 5	12 ± 1	521 ± 15	22 ± 0	99 ± 6	16 ± 0	694 ± 18