A thermostable bacterial cocaine esterase rapidly eliminates cocaine from brain in nonhuman primates

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A thermolabile bacterial cocaine esterase (CocE) has been identified that rapidly degrades cocaine with a $K_M$ of 1.33 $\pm 0.085 \mu M$. In vivo evaluation of CocE has shown protection against convulsant and lethal effects of cocaine in rodents, confirming the therapeutic potential of CocE against cocaine overdose. However, the current study is the first to evaluate the effects of CocE on cocaine brain levels. Positron emission tomography neuroimaging of $[^{11}C]c$ocaine was used to evaluate the time course of cocaine elimination from brain in the presence and absence of CocE in nonhuman primates. Systemic administration of CocE eliminated cocaine from the rhesus-monkey brain approximately three times faster than control conditions via peripheral actions through attenuating the input function from blood plasma. The efficiency of this process is sufficient to alleviate or prevent adverse central nervous system effects induced by cocaine. Although the present study used tracer doses of cocaine to access brain clearance, these findings further support the development of CocE for the treatment of acute cocaine toxicity.

MATERIALS AND METHODS

Subjects

Three adult rhesus monkeys (RGg-9, RZq-8 and RLa-10) weighing between 8.1–9.8 kg served as subjects. Each subject was housed individually and fed Purina monkey chow (Ralston Purina, St Louis, MO, USA), fruits and vegetables. Water was continuously available. Animal care procedures strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Emory University. All subjects received $[^{11}C]c$ocaine and two subjects (RGg-9 and RZq-8) also received $[^{11}C]R$TI-150.

Radioligands

Cocaine was obtained from the National Institute on Drug Abuse, Bethesda, MD, USA, and RTI-150 was obtained from Research Triangle Institute, Research Triangle Park, NC, USA. $[^{11}C]c$ocaine and $[^{11}C]R$TI-150 were synthesized using methods previously established by our laboratory. The chemical structures and labeling for each drug are shown in Figure 1.

CocE production

Plasmid expressing double mutant CocE (DM CocE) is described previously. Plasmid pET22bDMCocE was used to transform FreColi cells (RCT Technologies, Tucson, AZ, USA). Transformed cells were grown in TB media containing ampicillin antibiotic. Cells were induced with 1 mM IPTG.
[11C] Labeled Cocaine and RTI-150

\[
\begin{align*}
\text{nor-cocaine} & \rightarrow [\text{11C}]\text{cocaine} \\
\text{nor-RTI-150} & \rightarrow [\text{11C}]\text{RTI-150}
\end{align*}
\]

Figure 1. The chemical structures and labeling of [11C]cocaine and [11C]RTI-150.

Saline challenge

Esterase challenge

Figure 2. Sample PET images from a representative subject showing the uptake of [11C]cocaine and the subsequent elimination following saline (top) or CocE (bottom) challenge 10 min after administration of [11C]cocaine. The sequential images (from left to right) are shown in the horizontal plane. High density labeling in the caudate and putamen is easily identified. CocE, cocaine esterase; PET, positron emission tomography.

PET scanning

PET scanning was performed on a Siemens Focus 220 microPET scanner (Siemens, Concorde Microsystems, Knoxville, TN, USA). Animals were initially anesthetized with ketamine and Telazol, then intubated and maintained on 0.8–1.5% isoflurane for the duration of the imaging procedure. An acute venous catheter was placed in the saphenous vein for intravenous administration of the radioligand and the CocE or saline control. The catheter access was maintained with a sterile saline drip throughout the study. Animals were placed supine in the PET instrument, fitted with pulse oximetry equipment and a rectal thermistor for physiological monitoring during the procedure. Before injection of the PET radiopharmaceutical, a transmission scan was performed with a Co-57 point source to correct for attenuation in the image reconstruction. Approximately 300 MBq of the given radiopharmaceutical was injected over 5 min with an infusion pump in each animal. At 10 min post radiopharmaceutical injection, when maximal uptake was achieved, animals were challenged with either CocE (1.0 mg kg⁻¹) dissolved in saline solution or saline alone as a control. Images were reconstructed with OSEM/MAP using measured attenuation correction, zoom factor 2, and decay corrected to the time of injection.

Data analysis

Regions of interest (ROIs) were drawn manually on each animal's PET images fused with a rhesus MRI template over the caudate and putamen. An additional reference region was drawn over the cerebellum, which was assumed to have a negligible concentration of the dopamine transporter (DAT). The same regions were used for the saline and CocE challenges within the same animal. Regions of interest were then applied to all images to obtain time-activity curves and then normalized to the animal's body weight and injected activity.

The pharmacokinetics of [11C]cocaine uptake into the brain were simulated using mathematical compartmental analysis commonly applied in the quantification of blood flow, cerebral metabolism and neuroreceptor binding. Compartment representing physiological spaces or states of [11C]cocaine in the DAT-rich regions and in the esterase challenge are assigned to the (1) blood plasma, a space representing the amount of [11C]cocaine available to cross the blood brain barrier, (2) interstitial region, a space representing the free and nonspecific bound fraction of [11C]cocaine, and (3) bound state, representing [11C]cocaine that is specifically bound to the DAT. The plasma input concentration of [11C]cocaine is not explicitly known in these experiments, therefore it is estimated from the cerebellum which is assumed to have negligible specifically bound signal. The cerebellum can be modeled using only two compartments, (1) blood plasma, this is the same compartment as above and (2) reference region, representing the same compartment as interstitial region but in the cerebellum region. The use of a reference region for estimating the plasma input has been shown to be valid in the calculation of specifically bound signal in neuroreceptor studies. The addition of the interstitial and bound state compartments represents the observed PET signal in the imaging studies.

Kinetic analysis of the time-activity was performed with the General Reference Tissue Model, a modification of the Lammertsma model allowing for the initial compartment concentrations to be nonzero. The model rate constants describe a first-order exchange between compartments using the reference region as the plasma input function. The rate constants used in the model are: R—the ratio of plasma to extracellular constant of the striatum and reference region (K₁/K₃), K₃—the extracellular to plasma rate constant in the striatum (where, K₃ = K₅/R in the reference region), K₅—konBavail the DAT association rate (kon) times the number of available binding sites (Bavail)²⁻¹ and K₆—the dissociation rate from the DAT.

The model is solved by initially assigning values to the rate constants (R, K₁, K₃, K₅), comparing the model generated time-activity curve with the PET time-activity curve, calculating the least squares sum, then iteratively adjusting the rate constants such that the least squares sum is minimized. The minimization of the least squares is done in a computer environment (IDL 6.4, ITT Visual Information Solutions, Boulder, CO, USA). An estimate of available DAT was the solved rate constants calculated from the binding potential, BPₜ₉₀ = kon × Bavail/koff = k₃/k₅. K₅ (koff/kon) is assumed to be constant throughout the experiment; therefore BPₜ₉₀ represents the number of available DAT sites for binding with [11C]cocaine. In addition to the compartment model, the washout rate of [11C]cocaine from the striatum was calculated using time-activity data following the peak uptake and fitting that data with an exponential function and a constant (e(−λt)+C), where λ (1 min⁻¹) is the washout rate and C is a constant. A Mann–Whitney U test (nonparametric t-test) was used to compare statistically the washout rate between saline and CocE challenges.

Simulation data

It was hypothesized that there is a marked reduction in [11C]cocaine available in the plasma compartment to enter the brain interstitial space immediately following the esterase challenge. The pharmacokinetics of the rapid metabolism of [11C]cocaine were simulated by setting K₅, the rate constant describing the influx of [11C]cocaine to the brain, to zero at 10 min post radiopharmaceutical injection and at the time of esterase injection. The influx rate constants can be estimated directly from a two-tissue compartment model if the arterial input function is known. In the present case, the input function is the cerebellum, which is assumed to have a negligible concentration of DAT and therefore represents signal from the free interstitial space and blood.
Mean time-activity curves were created for the \([11\text{C}]\)cocaine CocE or saline control challenges by averaging the caudate and cerebellum time-activity curves from all three subjects. These averaged time-activity curves were fit with the General Reference Tissue Model to estimate the rate constants \((R, k_2, k_3, k_4)\). At 10 min, the DAT-rich compartment model was used to simulate the esterase challenge by assigning the compartment concentrations and rate constant calculated from the General Reference Tissue Model. In the DAT-rich compartment model, \(K_1\) was set to zero at 10 min, and was run from 10 min to 120 min to obtain the specifically bound compartment concentration over time. This simulation assumes that the introduction of the esterase compound does not alter the rate constants governing the binding of \([11\text{C}]\)cocaine to DAT \((k_3)\) or its elimination from brain \((k_2\) and \(k_4\)).

**RESULTS**

PET images for a representative subject (RGg-9) are shown in Figure 2. Note the high uptake of \([11\text{C}]\)cocaine in the caudate and putamen, two regions of interest with high DAT density. \([11\text{C}]\) cocaine binding diminished over 50 min following saline challenge. The reduction in \([11\text{C}]\)cocaine binding over time was markedly accelerated following CocE challenge, indicating rapid elimination of cocaine from brain.

Subsequent analyses quantified the pharmacokinetics of \([11\text{C}]\) cocaine binding. The regions of interest (caudate and putamen) and the reference region (cerebellum) were used to evaluate the time course for \([11\text{C}]\)cocaine uptake and elimination. Time-activity curves are shown for individual subjects in Figure 3, and rate

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**Table 1.** Rate constants for \([11\text{C}]\)cocaine uptake in brain during saline and esterase challenge

<table>
<thead>
<tr>
<th>Subject</th>
<th>Region</th>
<th>(k_1)</th>
<th>(k_2)</th>
<th>(k_3)</th>
<th>(k_4)</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RGg-9</td>
<td>Caudate</td>
<td>1.01</td>
<td>0.128</td>
<td>0.296</td>
<td>0.281</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>1.02</td>
<td>0.122</td>
<td>0.311</td>
<td>0.280</td>
<td>1.1</td>
</tr>
<tr>
<td>RGq-8</td>
<td>Caudate</td>
<td>1.05</td>
<td>0.412</td>
<td>0.354</td>
<td>0.237</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>0.90</td>
<td>0.412</td>
<td>0.316</td>
<td>0.211</td>
<td>1.5</td>
</tr>
<tr>
<td>RLa-10</td>
<td>Caudate</td>
<td>1.10</td>
<td>0.411</td>
<td>0.350</td>
<td>0.233</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>1.00</td>
<td>0.374</td>
<td>0.380</td>
<td>0.254</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Esterase challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RGg-9</td>
<td>Caudate</td>
<td>0.95</td>
<td>0.364</td>
<td>0.128</td>
<td>0.160</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>1.12</td>
<td>0.263</td>
<td>0.280</td>
<td>0.320</td>
<td>0.9</td>
</tr>
<tr>
<td>RGq-8</td>
<td>Caudate</td>
<td>1.16</td>
<td>0.763</td>
<td>0.403</td>
<td>0.302</td>
<td>1.3</td>
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<tr>
<td></td>
<td>Putamen</td>
<td>1.12</td>
<td>0.784</td>
<td>0.392</td>
<td>0.303</td>
<td>1.3</td>
</tr>
<tr>
<td>RLa-10</td>
<td>Caudate</td>
<td>0.93</td>
<td>0.502</td>
<td>0.431</td>
<td>0.299</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Putamen</td>
<td>1.10</td>
<td>0.552</td>
<td>0.460</td>
<td>0.292</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Abbreviation: BP, binding potential.
constants and BP_{ND} are reported in Table 1. The average time to peak levels of cocaine was 9.5 min, and cocaine levels dropped markedly after 40–50 min. CocE induced a significant increase in the elimination rate for cocaine ($P < 0.05$). The elimination rate for cocaine in caudate/putamen was 3.0 ± 0.7% per minute following saline and 8.0 ± 1.0% per minute following CocE. Hence, CocE induced approximately a three-fold increase in cocaine elimination from brain. There was no significant difference in BP_{ND} between the saline and esterase challenges in the caudate ($P = 0.47$) or putamen ($P = 0.51$), indicating that CocE-induced changes in the time-activity curves and brain elimination were exclusively due to reduced cocaine concentrations in blood. In contrast, the time to peak levels of [11C]RTI-150 was considerably greater, and drug levels were sustained for the duration of the 90-min session (Figure 4 and Table 2).

As predicted, CocE had no effect on the elimination rate of the cocaine analog RTI-150, which lacks the benzoyl ester linkage of cocaine.

Figure 5 shows the averaged time-activity curves and simulated model to demonstrate the consequence of $K_1$ going to zero immediately after esterase challenge, indicating the absence of [11C]cocaine in blood for transport to the brain. Note the elimination of cocaine in the simulation was slightly faster than the measured data suggesting that [11C]cocaine was not completely eliminated from the blood at the time of CocE injection but substantially reduced compared with saline.


displacement.

**DISCUSSION**

Cocaine overdose can result in profound cardiovascular and central nervous system alterations that are frequently lethal.$^{19}$ Nevertheless, standard care for cocaine overdose primarily

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**Table 2.** Rate constants for [11C]RTI-150 uptake in brain during saline and esterase challenge

<table>
<thead>
<tr>
<th></th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$k_3$</th>
<th>$k_4$</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RZq-8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>0.90</td>
<td>0.050</td>
<td>0.933</td>
<td>0.336</td>
<td>2.8</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.05</td>
<td>0.061</td>
<td>0.399</td>
<td>0.140</td>
<td>2.8</td>
</tr>
<tr>
<td>RGg-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>1.07</td>
<td>0.036</td>
<td>0.336</td>
<td>0.251</td>
<td>1.3</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.12</td>
<td>0.039</td>
<td>0.330</td>
<td>0.231</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Esterase challenge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RZq-8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>1.02</td>
<td>0.063</td>
<td>0.740</td>
<td>0.322</td>
<td>2.3</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.08</td>
<td>0.073</td>
<td>0.329</td>
<td>0.149</td>
<td>2.2</td>
</tr>
<tr>
<td>RGg-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>1.09</td>
<td>0.048</td>
<td>0.225</td>
<td>0.177</td>
<td>1.3</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.09</td>
<td>0.048</td>
<td>0.221</td>
<td>0.174</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Abbreviation: BP; binding potential.
enzymes, such as cocaine hydrolase\textsuperscript{24} that accomplish similar
ment. This model can be adapted to other mechanisms and other
paper using PET neuroimaging and compartment modeling. The
the brain. CocE-induced changes in the rate of \textsuperscript{[11C]} cocaine
indicated that, when administered alone, cocaine had a half-life
CocE for the treatment of acute cocaine toxicity

\textbf{CONFLICT OF INTEREST}

The authors declare no conflict of interest.

\textbf{ACKNOWLEDGMENTS}

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bacterial cocaine esterase (CocE): an in vivo study of CocE-mediated cocaine

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Simulated_Time-Activity_Curves}
\caption{Averaged time–activity curves and simulated model fit for K1 set to zero. The model curve represents the simulated bound fraction of \textsuperscript{[11C]} cocaine plus the cerebellum curve after esterase challenge. The decrease in the model time–activity curve is faster than the measured data suggesting that \textsuperscript{[11C]} cocaine was not completely eliminated from the blood at the time of CocE injection but substantially reduced compared with the saline challenge chase. CocE, cocaine esterase.}
\end{figure}
