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Acute cocaine administration alters permeability of blood-brain barrier in freely-moving rats— Evidence using miniaturized fluorescence microscopy

Jeffrey L. Barr^a, G. Cristina Brailoiu^b, Mary E. Abood^a, Scott M. Rawls^a, Ellen M. Unterwald^a, Eugen Brailoiu^a

^aCenter for Substance Abuse Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140, USA

^bDepartment of Pharmaceutical Sciences, Jefferson College of Pharmacy, Thomas Jefferson University, Philadelphia, PA 19107, USA

Abstract

Background—Cocaine has a variety of negative effects on the central nervous system, including reports of decreased barrier function of brain microvascular endothelial cells. However, few studies have directly shown the effects of cocaine on blood-brain barrier (BBB) function *in vivo*. The miniature integrated fluorescence microscope (i.e., miniscope) technology was used to visualize cocaine-induced changes in BBB permeability in awake, freely-moving rats.

Methods—The miniscope was implanted in the prefrontal cortex of adult male rats. After recovery and acclimation, rats received an injection of cocaine (5–20 mg/kg ip) 15 minutes following iv infusion of sodium fluorescein, a low molecular weight tracer. Fluorescence intensity was recorded *in vivo* via the miniscope for 30 minutes or 24 hours post cocaine administration and served as an indicator of BBB permeability.

Results—Results demonstrate that cocaine increased the sodium fluorescein extravasation in brain microcirculation in a dose-dependent manner 30 minutes, but not 24 hours after administration.

Conclusion—We report for the first time using direct visualization of brain microcirculation with the miniscope technology in awake, freely-moving rats, that acute cocaine administration produced a transient increase in the BBB permeability.

Correspondence: Ellen M Unterwald, Center for Substance Abuse Research, Lewis Katz School of Medicine at Temple, University, Philadelphia, PA 19140, USA, ellen.unterwald@temple.edu.

Contributors

EB, EMU and JLB designed the study; EB and JLB performed the experiments; GCB, MEA and SMR analyzed data; all authors participated in the writing of the manuscript and approved the final version.

Conflict of Interest

No conflict declared.

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Keywords

Cocaine; Blood-Brain Barrier; Sodium Fluorescein; Rat

1. Introduction

The blood-brain barrier (BBB) is a functional and structural interface that separates the circulation from the central nervous system and ensures the maintenance and regulation of the neural microenvironment (Hawkins and Davis, 2005). The BBB allows the transport of nutrients, removal of metabolites, and protection of the brain (Abbott et al., 2010). BBB permeability is mainly controlled by the monolayer of endothelial cells connected via tight and adherens junctions (Hawkins and Davis, 2005).

Cocaine remains a major drug of abuse, and reports indicate that cocaine can alter the BBB permeability via multiple mechanisms (Sharma et al., 2009). In brain microvascular endothelial cells cocaine, similarly to HIV, down-regulates tight junction proteins (Dhillon et al., 2008; Toborek et al., 2005). Cocaine alters expression of intracellular adhesion molecule 1, vascular cell adhesion molecule 1, endothelial-leukocyte adhesion molecule, and promotes neuro-inflammation (Fiala et al., 2005; Fiala et al., 1998; Kousik et al., 2012). Other studies indicate that cocaine acts via Sigma-1 receptors (Narayanan et al., 2011), modulates brain endothelial cell function by inhibiting store-operated calcium entry (Brailoiu et al., 2016; Rosado, 2016) and increases endothelial permeability (Yao et al., 2011). The effect of cocaine on the BBB contributes to potentiation of HIV-associated neurocognitive disorders (Cai et al., 2016; Fiala et al., 2005; Kousik et al., 2012). More recently, cocaine has been reported to impair the BBB permeability by increasing the expression and activity of prolydase that catalyzes collagen degradation during BBB remodeling (Ysrayl et al., 2019).

These studies provide important evidence that cocaine disrupts BBB function and suggest potential mechanisms involved. A limitation to these reports is that they were performed using *in vitro* cell systems. We used a miniature integrated fluorescence microscope (i.e., miniscope) to visualize in real-time cocaine-induced changes in BBB permeability in awake rats.

2. Materials and Methods

2.1 Animal Subjects

Adult male Sprague Dawley rats (Charles River Laboratories Inc.) were housed on a 12-hour light/dark cycle with free access to chow and water. Animal protocols were approved by the Institutional Animal Care and Use Committee of Temple University. The study followed the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines and the National Institutes of Health guide for the Care and Use of Laboratory Animals.

2.2 Chemicals

Cocaine was supplied by the National Institute on Drug Abuse's Drug Supply Program. Cocaine was dissolved in saline and injected ip in a volume of 1 mL/kg body weight. Sodium fluorescein (Sigma–Aldrich, St. Louis, MO), was dissolved in saline and injected iv.

2.3 Surgical Procedures and Assessment of BBB Permeability

Surgical methods were as described previously (Barr et al., 2015). Briefly, rats were anesthetized with isoflurane, and imaging cannula (Miniscope GRIN lens, Doric Lenses, Inc., Quebec, Canada) implanted into the prefrontal cortex using the following stereotaxic coordinates from bregma (AP: 3mm, ML: 0.5 mm, DV: 2.6 mm) (Paxinos and Watson, 2013). After two weeks of recovery and habituation experiments began.

2.3.1 Experiment 1. Cocaine Dose-Response on BBB Permeability—Sodium fluorescein (Na-F; 200 mg/kg iv) was injected through the tail vein followed 15 min later by cocaine (5–20 mg/kg, ip) or saline. Na-F extravasation, as an indicator of BBB permeability, was measured immediately prior to (i.e., baseline) and again 3–30 min following saline or cocaine. Fluorescence intensity (Ex/Em 488/520 nm) was measured in 10 regions of interest (ROIs) in close proximity of microvessels. The same 10 ROIs were recorded prior to and following cocaine administration using a within-subjects design. Fluorescence was visualized, recorded and analysed post-acquisition using Doric Studio software (Doric Lenses, Inc).

2.3.2 Experiment 2. Long-Term Cocaine Effects on BBB—This study was performed to determine if the effects of cocaine on BBB permeability were present 24 hours after cocaine administration. Rats were injected with Na-F and fluorescence was measured with the miniscope 15 min later in order to obtain baseline measurements for 10 ROIs in close vicinity of the microvessels. Fluorescence was measured again 48 hours later when it was no longer detected. Cocaine (20 mg/kg, ip) or saline was administered, and 24 hours later, Na-F was injected and fluorescence (intensity of the same 10 ROIs) was assessed 15 min later.

2.4 Data Analysis

A within-subjects design was used wherein fluorescence intensity following saline or cocaine was compared with fluorescence intensity immediately prior to the injection (baseline), and reported as percent of baseline. For Experiment 1, two-way repeated measures ANOVA (with one factor repetition) was used to assess significant differences between treatment groups, followed by Tukey's multiple comparisons post-hoc tests. A t-test was used to assess significance for Experiment 2.

3. Results

3.1 Cocaine Induces a Dose-Dependent Increase in Sodium Fluorescein Extravasation (Exp. 1)

Brain microcirculation in the prefrontal cortex was visualized with the miniscope following Na-F administration; Na-F extravasation was quantified as a measure of BBB permeability.

Cocaine produced a time- and dose-dependent increase in Na-F extravasation as compared with saline administration (Fig. 1A; $n=5$ rats/dose). Two-way repeated measures ANOVA showed a significant effect of cocaine on Na-F extravasation ($F_{(3, 16)}=195.0$, $P<0.0001$), a significant effect of time ($F_{(7, 112)}=265.3$, $P<0.0001$) and a significant interaction ($F_{(21, 112)}=51.51$, $P<0.0001$). Post-hoc analysis showed that at the cocaine 20 mg/kg dose, Na-F fluorescence was higher than that following saline ($P<0.01$), 5 mg/kg cocaine ($P<0.01$) and 10 mg/kg cocaine ($P<0.05$) at time points 5–30 min. Cocaine 10 mg/kg significantly increased Na-F extravasation at 10–30 min post injection as compared to saline and 5 mg/kg cocaine ($P<0.01$). Cocaine 5 mg/kg was not significantly different than saline ($P>0.05$) and there were no significant differences at 3 min post-cocaine. Examples of Na-F fluorescence immediately before (control baseline) and 30 min after injection of saline or cocaine (20 mg/kg) are shown in Fig. 1B. Pseudocolor images are shown in Fig. 1C. Cocaine increased Na-F extravasation, indicating leakage of Na-F from the vasculature and an increase in BBB permeability.

3.2 Cocaine Produces a Transient Increase in Sodium Fluorescein Extravasation (Exp. 2)

Baseline Na-F extravasation was quantified and, using a within-subjects design, miniscope imaging was repeated 48 hours later (Fig 2). As expected based on the clearance of Na-F, no fluorescence was detectable 48 hours later (Fig. 2). Cocaine (20 mg/kg ip) was administered, followed 24 hours later by Na-F and miniscope imaging repeated. Extravasation of Na-F 24 hours after cocaine administration was not significantly different from baseline Na-F extravasation ($P=0.215$; Fig. 2). This result indicates the transient effect of a single dose of cocaine on BBB permeability which lasts less than 24 hours.

4. Discussion

Changes in BBB permeability in response to several drugs and conditions have been characterized using *in vitro* models (Deli et al., 2005). *In vivo* studies of BBB permeability assess extravasation of endogenous tracers or injected molecules that cannot cross an intact BBB (Wunder et al., 2012). Evans Blue (MW 961 Da) and Na-F (MW 376 Da) are commonly used for this purpose (Yen et al., 2013), and their accumulation in the brain is assessed *ex vivo* (Brailoiu et al., 2018; Leo et al., 2019). *In vivo* two/multiphoton laser scanning microscopy allows the high-resolution dynamic imaging of BBB (Nishimura et al., 2006). While this technique permits time-lapse BBB imaging and correlation with neuronal responses, it is performed in anesthetized animals (Zhang and Murphy, 2007).

The miniscope is a transformative technology developed to assess neuronal function *in vivo* (Ghosh et al., 2011). An important advantage of examining the BBB permeability with the miniscope over classical methods is the higher anatomical selectivity, as fluorescent tracer extravasation is examined at selected ROIs in close proximity to the brain microvessels. Importantly, the miniscope technique allows the assessment of fluorescent tracer extravasation in real-time in freely-moving animals thus avoiding any potential effect of the anesthetic. Several anesthetic agents can impact BBB function in rodent models of neurotrauma (Sharma et al., 2019).

We assessed the effect of cocaine on the BBB permeability *in vivo* using miniscope, as previous reports suggest that cocaine may impair the barrier function (Sharma et al., 2009). We found a time- and dose-dependent increase in Na-F extravasation following acute cocaine administration, indicative of increased BBB permeability. Of note, Na-F fluorescence was undetectable when re-imaged 48 hours later which is in agreement with the reported pharmacokinetics of low molecular weight fluorescein-conjugated dextrans (Mehvar and Shepard, 1992). Na-F extravasation 24 hours after cocaine administration was not different from pre-cocaine baseline measurements. Previous studies indicate that cocaine is undetectable in the plasma 24 hours after injection (20 mg/kg) (Nayak et al., 1976). Since we identified that cocaine increased Na-F extravasation 5–30 minutes after administration but not 24 hour later, we conclude that the effect of a single injection of cocaine on BBB permeability is transient. It will be important in future studies to determine if chronic cocaine administration damages BBB function in a sustained manner.

5. Conclusions

Here we provide the first evidence that acute cocaine administration produces a transient increase in the BBB permeability in freely moving rats. Loss of barrier function may increase cocaine-induced neurotoxicity perhaps through enhanced cellular stress or edema, or by permitting increased entry of toxins or microbes into the CNS, supporting the need for further investigation of mechanisms involved in changes in BBB in the presence of cocaine.

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Abbreviations

BBB	blood-brain barrier
miniscope	miniature integrated fluorescence microscope
Na-F	sodium fluorescein
ROIs	regions of interest

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Highlights

- The effect of cocaine on BBB was examined using miniscope implanted in adult rats.
- Cocaine transiently increased Na fluorescein extravasation in brain microvessels.
- In awake rats cocaine increased BBB permeability in dose- and time-dependent manner.

Figure 1. Cocaine induces a time- and dose-dependent increase in sodium fluorescein (Na-F) extravasation.

A, Na-F extravasation is shown over time after injection of saline or cocaine (5, 10 and 20 mg/kg, ip). Cocaine (10 and 20 mg/kg) produced an increase in Na-F extravasation. Data are expressed as average fluorescence intensity ($\pm$ SEM) for 10 ROIs, normalized to control baseline fluorescence measured immediately prior to injection of saline or cocaine in the same subject. (*) $P < 0.01$ as compared with saline; (#) $P < 0.05$ compared with cocaine 10 mg/kg or $P < 0.01$ compared with cocaine 5 mg/kg. $N = 5$ rats per treatment group. **B**,

Examples of autofluorescence before Na-F injection and of Na-F fluorescence visualized with miniscope in rat prefrontal cortex before (control baseline) and 30 min after injection of saline (top) or cocaine (20 mg/kg, bottom). **C**, Pseudocolor images with calibration bar of images shown in panel B. Examples of 2 ROIs are indicated in panels B and C.

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Figure 2. Cocaine had no effect on sodium fluorescein (Na-F) extravasation 24 hours after administration.

A, Summary of the experimental design. **B**, Comparison of Na-F extravasation in control (baseline Na-F) and 24 hours after a single injection of cocaine (20 mg/kg ip). Baseline Na-F extravasation was determined by averaging the fluorescence intensity for 10 ROIs in the vicinity of microvessels 15 min after iv administration of Na-F. Extravasation of Na-F 24 hours after cocaine was not significantly different ($P = 0.215$) from baseline Na-F extravasation. Data are expressed as average fluorescence intensity ($\pm$ SEM) after normalization to control baseline fluorescence; $N=5$ rats. **C**, Examples of pseudocolor images with calibration bar.