Introduction

Sustained abstinence from cocaine use is frequently compromised by exposure to stimuli that have previously been associated with drug taking. Such cues trigger drug-associated memories leading to craving and relapse. Our previous work has shown that altering cocaine-cue memories by interfering with the reconsolidation process is a potential therapeutic tool to prolong abstinence. We have previously shown that the histone acetyltransferase (HAT) inhibitor, garcinol, can impair the reconsolidation of cocaine-cue memories in a manner that is reactivation specific, temporally constrained, and long lasting (Monsey MS, Sanchez H & Taylor JR, Neuropsychopharmacol, 42(9):587-597).

Here, we examined the neuroproteomic profile in the lateral nucleus of the amygdala (LA) following cocaine-cue memory retrieval and systemic garcinol administration. We further examined the downstream effects of garcinol using primary neuronal cultures and cell lines.

Materials & Methods

*Adult male Sprague-Dawley rats, weighing 250–300 g, were housed individually in a 12-h light/dark cycle. Throughout the experiment food was restricted to maintain rats at 90–95% of their body weight. All experiments were conducted in compliance with institutional guidelines. Injections were performed on the day of surgery or 15 minutes prior to cocaine injection.

**Surgical procedures:** Rats were anesthetized with 7% halothane anesthesia and 2% halothane isoflurane vapor. They were orally intubated to receive a solution containing 2% halothane, 7% isoflurane, and 100% oxygen. The solution was administered with a microinfusion pump at a rate of 0.3 mL/min. Each animal was checked for breathing and heart rate to ensure adequate anesthesia. A DA record was maintained, and any animal that did not meet the criteria was excluded.

**Histological procedures:** Rats were anesthetized with 7% halothane anesthesia and 2% halothane isoflurane vapor. They were orally intubated to receive a solution containing 2% halothane, 7% isoflurane, and 100% oxygen. The solution was administered with a microinfusion pump at a rate of 0.3 mL/min. Each animal was checked for breathing and heart rate to ensure adequate anesthesia.

**Protein extraction:** LA tissue was collected for proteomic analysis using a tissue-free quantitative approach (TFQ-MW). Samples were processed and extracted in lysis buffer (2% CHAPS in water) with an equal amount of RT-qPCR standard template in a ratio of 1:10 (standard/sample) on all samples. Extracted proteins were digested with trypsin (Promega) and analyzed in a custom (QqQ) mass spectrometer (Thermo Fisher Scientific). The protein content was determined by bicinchoninic acid (BCA) assay, and the peptide content was determined by enzyme-linked immunosorbent assay (ELISA).

**Primary cell culture:** Primary hippocampal, striatal, and cortical neurons were cultured and maintained for 14 days. This protocol is adapted from Backstrom et al. (1986). The cells were grown in minimum essential medium (MEM) with 1% penicillin-streptomycin. On day 14, the neurons were treated with either DMSO (0.1%) or vehicle (0.1% DMSO) in MEM. Following treatment, the cells were fixed with 100% methanol for 10 min at −20 °C, and stored at −80 °C until use. The images were analyzed using ImageJ or NIH Image software.

**Cell lines:** Human neuroblastoma SH-SY5Y cells (ATCC) were grown in Dulbecco’s Modified Eagle Medium (DMEM) with 1% heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% HSA, and 5% Horse serum. The cells were grown at 37°C in a humidified atmosphere of 5% CO₂.

Results

**Figure 1: Differential protein expression in the LA following cocaine-cue memory reactivation and garcinol administration**

**Figure 2: Garcinol decreases alpha-tubulin acetylation in primary hippocampal, striatal, and cortical neurons**

**Figure 3: Garcinol decreases alpha-tubulin acetylation in human neuroblastoma SH-SY5Y neurons**

**Figure 4: Garcinol decreases Fez1 protein expression in hippocampal and striatal primary neurons**

Conclusions

- Garcinol administration following cocaine-cue memory retrieval drives the differential expression of 14 proteins in the LA, half of which are associated with changes in microtubule dynamics.
- In primary hippocampal, striatal, and cortical neurons, as well as in human neuroblastoma SH-SY5Y neurons, garcinol decreases levels of alpha-tubulin acetylation at 5 minutes, but not at 15 or 30 minutes after administration.
- Garcinol additionally decreases levels of the kinesin adaptor protein, Fez1, protein expression in hippocampal and striatal primary neurons 15 minutes after administration.

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