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Cystine/glutamate exchange serves as the source for extracellular glutamate: Modifications by repeated cocaine administration*

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Summary. Repeated administration of cocaine lowers the basal extracellular levels of glutamate in the nucleus accumbens as measured by microdialysis. The studies presented reveal that this long-term neuroadaptation elicited by repeated cocaine results from a decrease in the activity of cystine/glutamate exchange.

Keywords: Cocaine – Cystine/glutamate exchanger – Microdialysis

Introduction

Repeated administration of cocaine produces a significant decrease in extracellular glutamate levels in the nucleus accumbens (NAc), as measured by microdialysis (Pierce et al., 1996). Surprisingly, however, the cellular mechanisms modulating extracellular glutamate levels have yet to be identified. Several cellular mechanisms that can modulate basal extracellular glutamate levels include vesicular release, nonvesicular release, and reuptake via Na-dependent transporters. A potential source for nonvesicular release of glutamate is via cystine/glutamate exchange, which has been demonstrated to release glutamate in tissue slices but has not been examined *in vivo* (Warr et al., 1999). The goal of the present study is to examine the contribution of these release and reuptake mechanisms to the maintenance of basal extracellular glutamate levels, and to characterize neuroadaptations in these cellular mechanisms following repeated cocaine administration.

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Methods

Experiment 1

The origin of extracellular glutamate was identified by determining the contribution of vesicular and nonvesicular release via cystine/glutamate exchange to extracellular glutamate levels in the NAc. Microdialysis probes aimed at the NAc were inserted into rats, and the next day, five 20-min baselines samples were collected. Additional samples were then collected with the Na^+-channel blocker tetrodotoxin (1.0 μM), the Ca^+-channel blockers ω-conotoxin GVIA (1 & 10 μM) plus diltiazem (10 & 100 μM), or the cystine/glutamate exchange blockers homocysteic acid (0.05–50 μM) and (S)-4-carboxyphenylglycine ((S)-4-CPG; 0.05–50 μM) added to the dialysis buffer. Glutamate content in the samples was then quantified using HPLC with fluorescence detection.

Experiment 2

The effect of cocaine on glutamate levels in the NAc was examined by determining extracellular levels of glutamate via no-net flux in rats withdrawn from daily saline or cocaine. Three weeks following daily treatment, microdialysis probes aimed at the NAc were inserted into rats, and the next day one of four concentrations of glutamate (0.6–10.67 μM) was added to the dialysis buffer. The no-net flux technique estimates extracellular levels by determining the concentration of glutamate in the buffer that produces no-net flux or diffusion across the dialysis membrane.

Experiment 3

The mechanism contributing to cocaine-induced diminished extracellular glutamate levels was determined by examining differences in release via cystine/glutamate exchange and reuptake via Na^+-dependent transporters. Three weeks following daily treatment, microdialysis probes aimed at the NAc were inserted into rats, and the next day cystine (0.01–0.3 μM), the cystine/glutamate exchange blocker (S)-4-CPG (0.0005–0.5 μM), or the Na^+-dependent reuptake blocker DL-threo-b-benzyloxyaspartate (TBOA; 10–1000 μM) was added to the dialysis buffer.

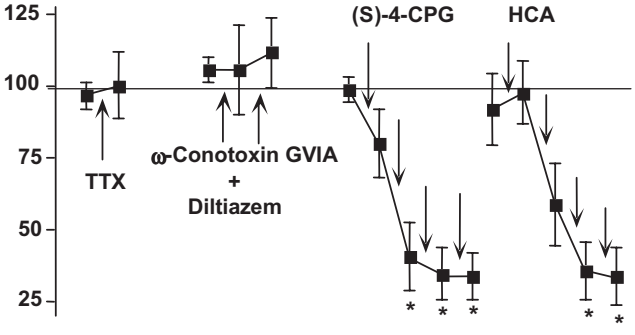

Fig. 1. Percent changes of basal values of extracellular glutamate levels in the NAc following reverse dialysis of tetrodotoxin (0, 1 μM; N = 9), conotoxin plus diltiazem (0, 1 & 10, 10 & 100 μM, respectively; N = 8), or cystine/glutamate exchange blockers (S)-4-carboxyphenylglycine (0, 0.05, 0.5, 5.0, 50 μMs; N = 7), and homocysteic acid (0, 0.05, 0.5, 5.0, 50 μM; N = 5). Arrow indicates an increase in drug concentration. Asterisk indicates a significant difference from baseline, ANOVA, $p < 0.05$

Results and discussion

Extracellular levels of glutamate measured by microdialysis in the NAc were found to be maintained primarily by the activity of cystine/glutamate exchangers. In support of this conclusion, the voltage-dependent sodium and calcium channel blockers TTX and conotoxin + diltiazem, respectively, failed to significantly alter extracellular levels of glutamate in the NAc, whereas both cystine/glutamate exchange inhibitors, (S)-4-CPG and homocysteic acid, produced a significant decrease.

Repeated cocaine administration produced a significant decrease in extracellular glutamate levels in the NAc (basal levels at 3 wks following repeated cocaine = 2.5 μM; following repeated saline = 5.7 μM), and this effect appears to be due to diminished release via cystine/glutamate exchange. Supporting this assertion, the infusion of cystine into the NAc in cocaine withdrawn rats increased extracellular glutamate levels to the level of the controls, while intra-accumbens infusion of cystine in saline withdrawn rats did not significantly alter extracellular glutamate levels. This suggests that the rate of cystine/glutamate exchange, mediated by intra- and extracellular substrate concentrations, is not modified by further increases in extracellular levels of cystine. As opposed to differences in glutamate release, there were no differences in Na^+-dependent glutamate reuptake since infusion of TBOA produced similar increases in cocaine and saline withdrawn rats. Collectively, these data indicate that basal extracellular glutamate levels in the NAc are maintained by cystine/glutamate exchange, and that this process is altered following repeated cocaine administration leading to lower extracellular levels.

References

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