Identification of Biochemical Changes in Cocaine-Treated PC12 Cells

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Introduction

Neuronal development, outgrowth, branching, and retraction play an important role in the process of neuronal networking at the embryonic and adult stages. Injury to neurons by an external insult, such as drug abuse, affects neuronal structure and networking system, or even causes neuronal death through inflammation.[1,2] We investigate the changes in structural integrity of neurons and their networking that result from cocaine treatment in rat pheochromocytoma PC12 cells by studying the level of biochemical markers that are associated with these changes. It is known that cocaine blocks re-uptake of dopamine into presynaptic dopaminergic neurons, resulting in accumulation of synaptic dopamine.[3] We propose that cocaine treatment will also increase lactate production indicating hypoxic state in cells. We study lactate levels as a marker to study how cocaine impact cells by causing significant loss in the neuritis.

Experimental

We utilize solution-state NMR spectroscopy to monitor the lactate level in cells as a biochemical marker to study the cellular stress induced by the uptake of cocaine. Differential cells in 96-well plates are treated with differential dose of cocaine (2~4 mM) for 48 hours. The media from treatments and control were utilized for lactate studies. 700.0 μL of sample solution was transferred into a 5 mm glass tube by using an Eppendorf micropipet for solution-state NMR experiments. For applying ^{2}H lock for field stabilization 100.0 mL D_2O was also added into each sample tube. Additionally, 200.0 μL of 3.30 mM DMSO, whose peak appearing at 2.66 ppm in frequency does not overlap with any sample peaks, was added into the sample solution for the quantification of the amount of lactate in the sample. The dominant water peak at 4.69 ppm was suppressed by applying the WATERGATE pulse sequence.[4] In Fig. 1, lactate peaks are visible at 1.25 ppm for the methyl group (doublet) and 4.04 ppm for the methine group (quartet). The bigger methyl peak of lactate was utilized for the quantification of lactate in each sample solution by comparing it to the peak intensity of methyl groups of DMSO whose concentration in each sample is fixed.

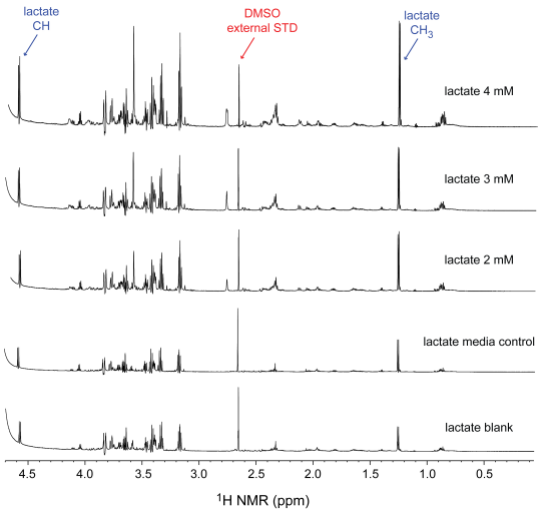

Fig.1 Lactate release in cell media by cocaine uptake investigated by ^{1}H NMR spectroscopy. After treating the cells with various concentrations of cocaine for 1 hour, the cell medium was taken for NMR measurement. Medium from the untreated cells was taken as a control, while the medium without cells was used as a blank.

Results and Discussion

From Fig. 1 it is obvious that approximately a 3-fold increase in lactate due to cocaine treatment was observed in the media (top spectrum; the lactate concentration obtained for each spectrum was indicated in the figure) compared to the spectrum obtained from the control cells (the second spectrum from the bottom). This result indicates that the cocaine treated cells experienced a hypoxic state where pyruvate was converted to lactate.

Conclusions

Cocaine-induced lactate release in cells was proven.

Acknowledgements

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References

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