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Simultaneous Analysis of Norepinephrine, Dopamine, and Serotonin in Microdialysis Perfusates Using HPLC-ECD

INTRODUCTION

The analysis of norepinephrine (NE) continues to be a problem, even more so when measured in conjunction with dopamine (DA) and serotonin (5-HT). Until now, numerous attempts to develop such a method have met with limited success. The usual approach requires use of multiple analytical systems, thereby wasting precious sample.

Neuroscientists using microdialysis perfusion continuously demand greater temporal and spatial resolution. This can be achieved by placing smaller probes in more discrete brain regions, and by increasing the sampling rate. However, the resulting low-volume and dilute samples can be challenging to analyze, requiring a highly sensitive analytical system.

Today, a single chromatographic system provides simultaneous quantitation of NE, DA, and 5-HT in a single sample. This makes more efficient use of each sample and lets researchers get information about multiple pathways in a single analysis. It also provides a superior method for NE measurement.

This work presents an example of how the method can be used to measure prefrontal cortical levels of NE, DA, and 5-HT under basal and amphetamine stimulated conditions.

EQUIPMENT AND CONDITIONS

Liquid Chromatography

An isocratic high-performance liquid chromatography (HPLC) system similar to the Thermo Scientific Dionex UltiMate® 3000 system including a pump, autosampler, and Thermo Scientific ESA Coulochem® III detector

Coulochem III Organizer Module with Temperature Control (P/N 70-9121TA)

Column: MD160, 1.5 × 250 mm, 5 µm
(P/N 70-7277)

Mobile Phase:¹ MD-3MA

Flow Rate: 0.20 mL/min

Temperature: 32 °C

Inj. Volume: 10 µL (partial loop)

Detector

Coulochem III (P/N 70-9143)

Analytical Cell (P/N 70-4131)

Guard Cell (P/N 55-0417)

Cell Potentials: Analytical Cell, E = +220 mV
Guard Cell, EGC = +275 mV

Microdialysis

Probe: CMA11, 3 mm membrane length
 Perfusion
 Flow Rate: 2.0 $\mu\text{L}/\text{min}$
 Artificial Cerebrospinal Fluid (aCSF) Composition:
 Sodium: 147 mM
 Potassium: 2.7 mM
 Calcium: 1.2 mM
 Magnesium: 1.0 mM
 Chloride: 150 mM
 Phosphate: (pH 7.4 + 2) 2 mM
 Collection Period: 20 min into 10 μL 0.2 M perchloric acid (containing 0.2 μM ascorbic acid and 0.2 μM EDTA)
 Animal Model: Anesthetized rat
 (urethane: 1.5g/kg i.p.)
 Post Surgery
 Recovery: 2–4 h
 Probe Coordinates: AP +3.2, LR +0.8, DV –5.0
 (from Bregma)

RESULTS AND DISCUSSION

This method resolved all three monoamines in under 16 min (Figure 1), showed excellent linearity (Figure 2), and extreme sensitivity (~ 100 – 150 fg on column).

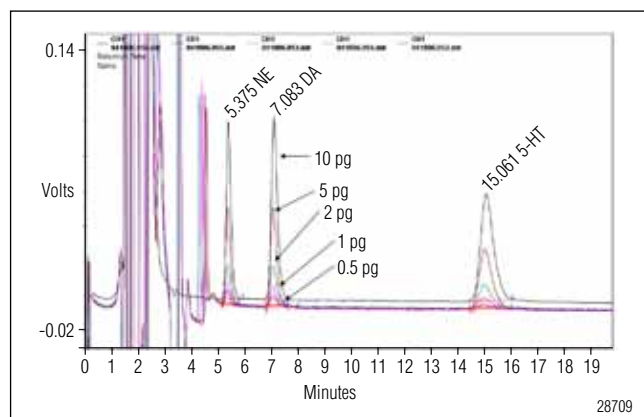


Figure 1. Chromatography of NE, DA, and 5-HT standards in aCSF.

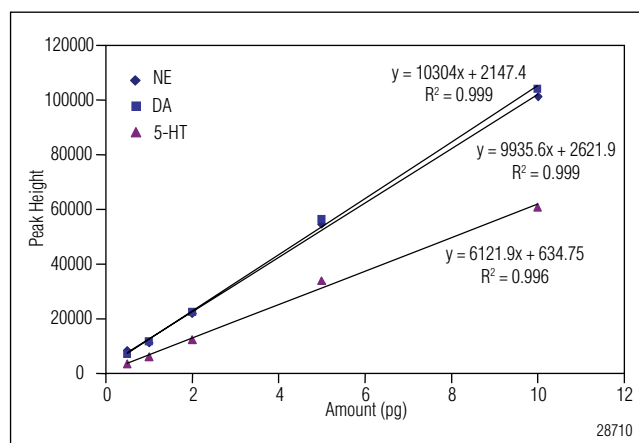


Figure 2. Linear curves for NE, DA, and 5-HT.

The method simultaneously measured all three monoamines under basal conditions, even in the urethane anesthetized animal where levels are expected to be depressed (Figure 3). Typical basal levels (uncorrected for in vitro recovery) were ~ 500 , 250 , and 160 fg/ $10 \mu\text{L}$, for NE, DA, and 5-HT, respectively (data not shown).

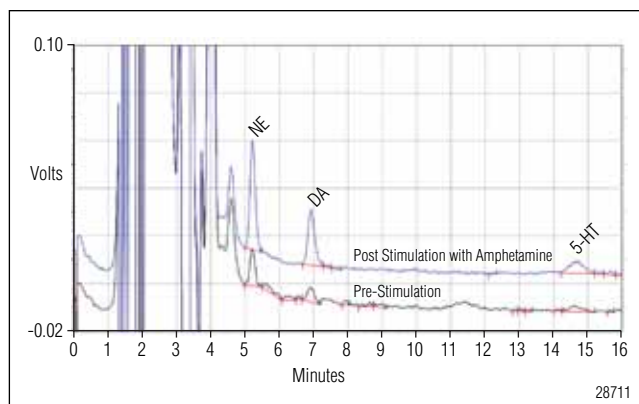


Figure 3. Microdialysis samples obtained from a 3 mm concentric microdialysis probe placed in the prefrontal cortex of an anesthetized rat, before and after amphetamine (2 mg/kg i.p.) stimulation.

Stimulation with amphetamine (2 mg/kg i.p.) resulted in significant increases of all three monoamine neurotransmitters to ~1500, 960, and 290 fg/10 μ L, for NE, DA, and 5-HT, respectively (Figure 3).

This method will be of great benefit to any researcher interested in studying the regional interplay of monoamine neurotransmitters during pharmacological manipulation or behavior.

ACKNOWLEDGEMENT

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REFERENCES

1. Dionex Corporation, *Simultaneous Analysis of Monoamines*. Technical Note 70-7661. Chelmsford, MA.

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