ANTEC LEYDEN

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MHPG and noradrenaline in rat preoptic area dialysate

Introduction

Analysis of the catecholamines in brain, peripheral tissues and body fluids has resulted in a more than basic understanding of the normal and disturbed peripheral sympathetic and central nervous systems in man and experimental animals. Quantitative analysis of the various metabolites has been at least equally important for the understanding of the neurodynamics of catecholamines.
Each of the catecholamines can be metabolised by the enzymes catechol-O-methyltransferase (COMT) and monoamineoxidase (MAO). Major metabolites of adrenaline are metanephrine and vanillylmandelic acid (VMA). Dopamine metabolites include homovanillic acid (HVA) and 3-methoxytyramine (3-MT). Noradrenaline metabolites include normetanephrine, VMA and 3-methoxy-4-hydroxyphenylglycol (MHPG). MHPG is considered to be (almost) exclusively of central nervous system origin.
This application describes the determination of noradrenaline and its metabolite MHPG after microdialysis sampling in rat preoptic area.

Method

Microdialysis sampling is accomplished by implanting a small dialysis membrane into living tissue. The membrane is integrated into a probe which is flushed by an isotonic perfusion fluid, at a constant flow rate. The difference between the concentration of a chemical in the tissue and concentration in the perfusion fluid creates a concentration gradient which drives this analyte across the dialyzing membrane. Since diffusion is bi-directional, the same device may either deliver or sample chemicals in the targeted tissue.
In combination with microdialysis the DECADE offers the possibility of fully automated analysis with on-line sample injection. A dialysis probe is on-line connected with an automated injector in the DECADE. In the 'auto mode' dialysates are analysed by automatically switching the injection valve and starting the data system.

LC-EC conditions

Column	Supelco LC-18-DB, 150 x 4.6 mm, 3 µm
Flow rate	1.0 ml/min
Mobile phase	75 mM NaH_2PO_4, 0.1 mM EDTA, 0.55 mM OSA, pH 4.95, 7.5% methanol
Sample	Rat preoptic area dialysate, 50 µl inj. (30 µl sample + 20 µl, 25 mM HAc, off-line sampling)
Temperature	35 °C
E-cell	650 mV (vs. Ag/AgCl, sat'd)

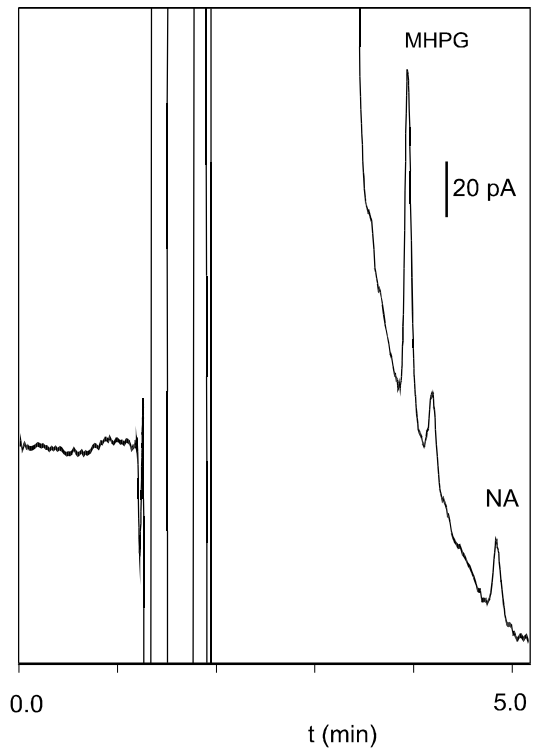

Fig. 1. Analysis of rat preoptic area dialysate. Concentrations (amounts) are 10 nM (318 fmol) MHPG and 0.36 nM (11 fmol) NA. Courtesy: Astrid Linthorst, Max Planck Inst. Psychiat., Clin. Inst., Munich, Germany.

Part numbers and configuration used

120.0035	DECADE EC detector
110.4105	VT03 flow cell with 3mm electrode and salt bridge Ag/AgCl REF

Configuration suggestions & comment

Replacement of the REF by an ISAAC-based flow cell (p.n. 110.4205) or use of an ISAAC REF block (p.n. 110.6205) makes the flow cell practically maintenance free. The ISAAC requires 2 mM KCl in the mobile phase. In case of microdialysis samples 8 mM KCl must be added and the E-cell must be adapted in both cases.
The programmable DECADE II (p.n. 171.0035) with its superior electronic noise suppression (ADF) and temperature stability will provide improved performance.