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Original article

Determination of dopamine concentrations in brain extracellular fluid using microdialysis with short sampling intervals, analyzed by ultra high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

Introduction: An increase in striatal dopamine is considered essential for the rewarding and reinforcing effects of drugs of abuse. We have developed and validated an ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method for the analysis of dopamine in rat brain extracellular fluid (ECF) sampled with microdialysis. The method was applied to monitor changes in dopamine concentrations over time after an intravenous bolus injection of heroin.
Methods: Dopamine and dopamine-d3 were analyzed using a 2.1 × 100 mm Aquity T3 column, 1.7 μm particle size, with a formic acid and methanol gradient. The run time of the method was 2.5 min including equilibration time.
Results: The method had an LOQ of 0.15 ng/mL, which equals 0.55 pg on column. The calibration curves were linear in the tested area of 0.15 to 16 ng/mL. Inter-assay coefficients of variation varied between 5–17%, with an accuracy expressed as bias of −10 to 5%. The intra-assay coefficients of variation varied between 9–15%, with an accuracy of −3–7%.
Discussion: Heroin metabolism is very rapid. Sampling intervals of only 2 min were thus required to obtain an adequate number of samples of dopamine analysis accompanying the concentration–time profile of opioids in the brain. Applying a flow of 2 μL/min, 4 μL of dialysate were sampled at 2 min intervals, in 7 μL internal standard. The injection volume onto the UPLC column was 10 μL.
Analyses of microdialysate samples from a rat given heroin i.v. showed that it was possible to measure baseline levels and rapid changes in dopamine concentrations with very short sampling periods.

1. Introduction

Microdialysis is a common method for measuring free concentrations of drugs in various tissues, both in animals and humans. It is an ideal technique for measuring compounds of interest in brain extracellular fluid (ECF) (Hammarlund-Udenaes, Bredberg, & Fridén, 2009; Gottas et al., 2012), and can therefore be used as a tool to monitor endogenous levels of neurotransmitters and their metabolites after systemic drug administration (Zetterstrom, Sharp, Marsden, & Ungerstedt, 1983; Li, Peris, Zhong, & Derendorf, 2006).

An increase in dopamine release in brain striatum after administration of drugs of abuse, is considered essential for their acute as well as long term effects, e.g., reward/saliency and reinforcing effects (for reviews see Pierce & Kumaresan, 2006; Volkow, Fowler, Wang, Swanson, & Telang, 2007). The analysis of neurotransmitters in microdialysate is commonly performed with liquid chromatography (LC) or capillary electrophoreses (CE). For neurotransmitters like dopamine, the use of mass spectrometric (MS) or electrochemical detection (ECD) for quantification has been common (Zhang & Beyer, 2006; Bicker, Fortuna, Alves, & Falcão, 2013). However Hows et al. (Hows, Lacroix, Heidbreder, Organ, & Shah, 2004) demonstrated that the level of dopamine measured by ECD could be overestimated due to artifacts, suggesting MS as a more suitable detector. Several LC–MS/MS or UHPLC-MS/MS methods for dopamine have recently been published (Syslova et al., 2011; Cannazza et al., 2012; Nirogi et al., 2013).

Abbrevations: liquid chromatography, (LC); capillary electrophoreses, (CE); electrochemical, (ECD); mass spectrometric, (MS); ultra high performance liquid chromatography tandem mass spectrometry, (UHPLC-MS/MS); extracellular fluid, (ECF); 6-monoacetyl morphine, (6-AM); morphine 3-glucuronide, (M3G); morphine 6-glucuronide, (M6G); quality control, (QC); multiple reaction monitoring transitions, (MRM transitions); Electrospray tandem mass spectrometry detection, (ESI-MS/MS-detection); limit of detection, (LOD); lower limit of quantification, (LLOQ); upper limit of quantification, (ULOQ); stable labeled isotope internal standards, (SIL-IS); coefficient of variation, (CV); intravenous, (i.v.).

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A major challenge in the analysis of microdialysate pertains to the combination of low sample volume and very low analyte concentrations (Zhang & Beyer, 2006). Sampling intervals of 20–30 min are therefore common to obtain sufficient amounts of dialysate and analyte for analysis (Vindenes et al., 2009; Syslova et al., 2011; Cannazza et al., 2012; Greco, Danysz, Zivkovic, Gross, & Stark, 2013; Nirogi et al., 2013). In on-going studies of heroin, our objective is to measure the relative role of heroin and its metabolites to the striatal dopamine overflow produced by i.v. heroin administration. Heroin metabolizes to 6-monoacetylmorphine (6-AM) and further to morphine, and is subsequently glucuronidated to morphine 3-glucuronide (M3G) and morphine 6-glucuronide (M6G) (for overview see (Rook, Huitema, van den Brink, van Ree, & Beijnen, 2006; Stowe et al., 2011)). Heroin and the primary metabolite 6-AM have very short half-lives, leading to rapidly changing concentrations of these opioids, as well as morphine, in the brain during the first 30 min after i.v. heroin administration (Gottas et al., 2013). Thus, very short sampling periods are necessary to be able to relate changes in striatal dopamine levels to the accompanying changes in ECF concentrations of heroin and its metabolites. Our aim was therefore to validate a method for dopamine measurement in rat brain ECF-dialysate sensitive enough for very short sampling periods.

2. Experimental

2.1. Chemicals and reagents

Dopamine and the internal standard dopamine-d3 were purchased from Fluka Chemika (Steinheim, Germany) and Chiron (Trondheim, Norway), respectively. Formic acid and LC-MS grade methanol were purchased from Merck (Darmstadt, Germany). De-ionized water was obtained from a MilliQ A10 water purification system (Millipore, Bedford, MA, USA).

2.2. Standard solutions

Two separate stock solutions were prepared in 0.1% formic acid in amber flasks, identified as calibration and quality control (QC). The internal standard was prepared in 0.1% formic acid. The stock solutions and internal standard were kept refrigerated. From the stock solutions fresh calibrators and QC-samples were prepared by dilution in Ringer's solution (148 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, 0.85 mM MgCl2) for each assay. The preparation of calibrators and QC's were performed on ice.

2.3. In vivo experiment

Surgical implantations were performed in accordance with previously established procedures in our laboratory (Gottas et al., 2012), with some modifications. In brief, the animals were implanted with two round-tip polyurethane catheters, one for injection of drug (femoral vein) and one for blood sampling (carotid artery). Two brain microdialysis guide cannulas (AT6.14.iC, AgnTho's, Lidingö, Sweden and CMA 12, CMA Microdialysis, Solna, Sweden) were implanted one on each side at the following coordinates relative to bregma: anterior (A): +0.5 mm; lateral (L): ±3.0 mm and lowered 4.0 mm ventrally into the striatum (Paxinos and Watson, 1998). About 18 h before the experiment, the microdialysis probes (AT6.14.4, AgnTho's, Lidingö, Sweden and CMA 12 Elite PAES, CMA Microdialysis, Solna, Sweden) were inserted into their respective guide cannulas. The standard CMA 12 was used for dopamine sampling. The microdialysis probes were perfused with Ringer's solution at 0.2 μl min^{-1} and the animal was left for acclimatization overnight. On the following day, the flow was increased to 2 μl/min, and the sample collection interval was set to 2 min. ECF-dialysate was sampled for about 20 min before injection of the drug to assess the baseline dopamine level. The rat then received an i.v. bolus injection (0.1 mL) of 3 μmol heroin (12.8 mg/mL), followed by 0.3 mL of a physiological saline solution to ensure a correct injection of the total heroin dose. Dialysis samples were collected for the subsequent 120 min.

The experimental protocol was approved by the Norwegian Animal Research Authority and carried out in concordance to Norwegian regulation and international standard procedures.

2.4. Sample preparation

Internal standard (7 μL) was dispensed into amber sample vials, to protect dopamine from light, before placing them in the fraction collector. The sampling time was 2 min, and the microdialysis flow was 2 μL/min. The fraction collector was cooled to 6 °C. The samples were transported to the MS-lab on ice. The samples were briefly mixed, and 10 μL was injected directly into the UHPLC-MS/MS system without further sample preparation. The temperature of the sample manager was 6 °C to enhance sample stability.

2.5. UPLC conditions

A Waters Acquity I-class UPLC module (Waters Corp., Milford, MA, USA) was used for separation. Gradient elution was performed on an Acquity HSS T3 column (2.1 × 100 mm, 1.7 μm) from Waters (Wexford, Ireland). A flow rate of 0.5 mL/min, with methanol (mobile phase A) and 0.1% formic acid solution (mobile phase B) as solvents, was used. The column temperature was held at 65 °C and the injection volume was 10 μL using partial loop injection. A pre-injection wash of 2 s and a post-injection wash of 6 s were used, with methanol as the purge solvent. The gradient was as follows: 100% B for 0.5 min, followed by a linear gradient to 100% A for 1 min, 100% A was kept for 0.3 min before returning to 100% B for 1.31 min. The total cycle time of the method was 2.5 min. The eluent was sent to waste for on-line desalting for 0.75 min at the initial part of the chromatographic run, and for elimination of potential late eluting endogenous interferences at 1.2 min.

2.6. MS/MS conditions

A Waters TQS tandem mass spectrometer (Waters Corp., Milford, MA, USA) was used for the analyses. ESI-MS/MS-detection was performed in the multiple reaction monitoring (MRM) mode using positive ionization. One transition was monitored for both dopamine and the internal standard, to get the best sensitivity possible.

The capillary voltage was set to 1.2 kV and the source block temperature to 150 °C. Nitrogen was used as desolvation gas, delivered at a temperature of 500 °C and a gas flow of 1000 L/h. The cone gas (nitrogen) was set to 150 L/h. Direct infusion into the MS was used to determine optimal MRM transitions, cone voltages and collision energies. Instrumental parameters (MRM transitions, cone voltages, collision energies and dwell times) used for the measurements are provided in Table 1.

System operation and data acquisition were controlled using MassLynx 4.1 software (Waters Corp., Milford, MA, USA). All data were processed with the TargetLynx quantification program (Waters Corp., Milford, MA, USA).

Table 1
Instrumental parameters.

	Transition	Dwell time (s)	Collision energy (eV)	Cone voltage (V)
Dopamine	153.9 > 91.0	0.05	28	20
Dopamine d3	156.9 > 94.0	0.05	28	20