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Monitoring of dopamine and its metabolites in brain microdialysates: Method combining freeze-drying with liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive assay method was developed for a parallel, rapid and precise determination of dopamine and its metabolites, homovanillic acid, 3-methoxytyramine and 3,4-dihydroxyphenylacetic acid, from brain microdialysates. The method consisted of a pre-treatment step, freeze-drying (lyophilization), to concentrate dopamine and its metabolites from the microdialysates, and a detection step using liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS). In particular, the reaction monitoring mode was selected for its extremely high degree of selectivity and the stable-isotope-dilution assay for its high precision of quantification. The developed method was characterized by the following parameters: the precision of the developed method was determined as ≥88.6% for dopamine, ≥89.9% for homovanillic acid, ≥86.1% for 3-methoxytyramine and ≥88.1% for 3,4-dihydroxyphenylacetic acid; the mean accuracy was determined as ≥88.2% for dopamine, ≥88.3% for homovanillic acid, ≥85.9% for 3-methoxytyramine and ≥88.6% for 3,4-dihydroxyphenylacetic acid. The developed method was compared to (1) other combinations of pre-treatment methods (solid phase extraction and nitrogen stripping) with LC–MS and (2) another detection method, liquid chromatography, with electrochemical detection. The novel developed method using combination of lyophilization with LC–ESI-MS/MS was tested on real samples obtained from the *nucleus accumbens* of rat pups after an acute methamphetamine administration. It was proven that the developed assay could be applied to both a simultaneous analysis of all four substrates (dopamine, homovanillic acid, 3-methoxytyramine and 3,4-dihydroxyphenylacetic acid) in microdialysis samples acquired from the rat brain and the monitoring of their slight concentration changes on a picogram level over time following methamphetamine stimulus.

1. Introduction

During the last decades it has become apparent from experimental studies that catecholamine neurotransmission is a focal point of research in neuroscience. In particular, dopamine (DA) is a major modulatory catecholamine in the brain involved in motor functions, mood, learning and reward. Furthermore, it plays an important role in several psychiatric disorders such as addiction, depression, schizophrenia and Parkinson's disease. The action of DA at the synapse is terminated by two main mechanisms: (1) DA is drawn back into the pre-synaptic neuron (reuptake) and recycled; (2) DA is sequentially transformed into metabolites in two metabolic phases. In the phase I of the biotransformation DA is metabolized enzymatically by catechol-*O*-methyltransferase (COMT) and/or by monoamine oxidase (MAO) to homovanillic acid (HVA), 3-methoxytyramine (3-MT) and 3,4-dihydroxyphenylacetic acid (DOPAC) (Fig. 1) [1]. The levels of DA metabolites produced in the phase I directly reflect the behavior of the dopaminergic system and provide vital information about the enzyme action and abnormalities [2]. Then, the metabolic phase II produces hydrophilic conjugates (glucuronides and sulfates) of DA and its phase I metabolites [3].

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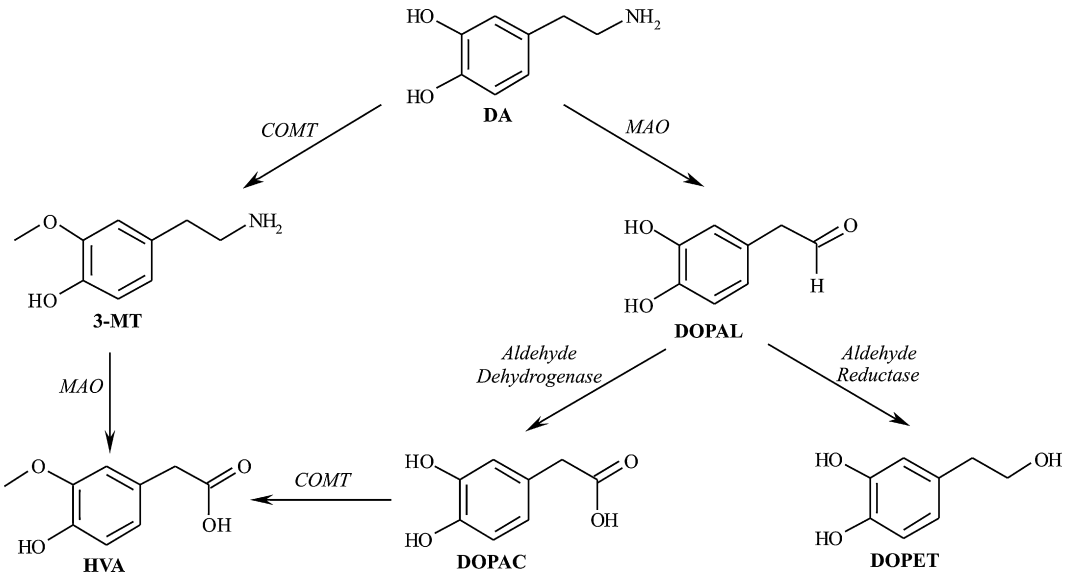

Fig. 1. Enzymes catalyzed metabolic pathways of dopamine: COMT = catechol-*O*-methyl transferase; MAO = mono amine oxidize; DA = dopamine; 3-MT = 3-methoxy-4-hydroxyphenethylamine; HVA = 3-methoxy-4-hydroxyphenylacetic acid; DOPAL = 3,4-dihyroxyphenylacetaldehyde; DOPAC = 3,4-dihydroxyphenylacetic acid; DOPET = 3,4-dihydroxyphenylethanol.

An acute administration of methamphetamine (MA) elevates extracellular levels of DA and 3-MT and decreases those of DOPAC and HVA [4,5]. Nevertheless, increased HVA levels after high MA doses have been also reported and thus the results are rather contradictory [6,7]. Some studies revealed that exposure to MA during pregnancy could impair the development of the neonatal central nervous system in rat pups [8].

The metabolism of neurotransmitters in the brain can be studied by microdialysis, where physiological perfusion fluid is pumped through a dialysis membrane that is surgically implanted into an animal brain region of interest [9]. The brain extracellular fluid contains synaptically released neurotransmitters and their metabolites, as well as compounds from non-synaptic sources [10]. These low molecular weight compounds in the extracellular fluid are extracted to the perfusion fluid by passive diffusion. The recovery of these compounds is dependent on many variables including temperature, molecular weight and charge, flow rate of the perfusion fluid and the surface area of the dialysis membrane [11].

Different analytical techniques have been employed to monitor DA and its metabolites (HVA, 3-MT, DOPAC) in biological samples, such as chromatography with fluorescence (FL), chemiluminescence (CL), ultraviolet (UV), electrochemical (EC) and mass spectrometry (MS) detection [10–14]. The most popular and successful technique widespread in the neurosciences has been high-pressure liquid chromatography (HPLC) coupled with electrochemical detection (ECD). Although this has long been the method of choice, it has disadvantages in the lengthy equilibration required and time-consuming analysis. Furthermore, analytes can only be identified by their retention times as is common with chromatographic methods. HPLC with mass spectrometry (HPLC–MS) is an alternative approach to clearly identify an analyte. The separation of analytes has commonly been achieved by reversed-phase liquid chromatography, utilizing an ion-pairing reagent. The derivatization of neurotransmitters has also been used to improve their separation and ensure sensitive detection by HPLC-FL as the concentrations of neurotransmitters in the brain microdialysates were extremely low (picomole and femtomole concentrations) [15]. Reversed-phase chromatography using volatile eluents has usually been used for HPLC of DA and its metabolites [16–18]. Since these polar analytes were only weakly retained on C18, a pentafluorophenylpropyl stationary phase was utilized to enhance the retention and achieve an adequate separation of the analytes from the inorganic salts of the artificial cerebrospinal fluid used in the microdialysis [3].

The present work deals with the development of a highly selective and precise analytical method for the simultaneous determination of DA and its metabolites (HVA, 3-MT and DOPAC) in brain microdialysis samples. The method consists of the pre-concentration step, lyophilization and LC–ESI-MS/MS detection. During the method development, three different preconcentrations were tested (lyophilization, solid phase extraction (SPE), nitrogen stripping) and compared. The methods were validated and compared with LC-ECD, the most frequently used method for the detection and quantification of neurotransmitters and their metabolites. The developed method (lyophilization and LC–ESI-MS/MS) was utilized for the analyses of *in vivo* samples from the *nucleus accumbens* collected after an acute MA administration. It was unambiguously demonstrated that the assay could be applied to the simultaneous analysis of all four analytes (DA, HVA, 3-MT and DOPAC) in microdialysis samples drawn from the rat brain and that it was sensitive enough for monitoring small changes in concentration over time.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents were of commercial origin: 3-hydroxytyramine hydrochloride (=dopamine hydrochloride; =DA·HCl, ≥99.0%; Sigma–Aldrich, USA); homovanillic acid (=HVA, ≥99.0%; Sigma–Aldrich, USA); 3,4-dihydroxyphenylacetic acid (=DOPAC, ≥99.0%; Sigma–Aldrich, USA); hydrochloric acid (37%; Sigma–Aldrich, USA); sodium chloride (99.5%; Sigma–Aldrich, USA); magnesium chloride (purum; Sigma–Aldrich, USA); calcium chloride (anhydrous, 93%; Sigma–Aldrich, USA); potassium chloride (purum; Sigma–Aldrich, USA); citric acid (≥99.5%; Sigma–Aldrich, USA); ethylenediaminetetraacetic acid (=EDTA, 99.99%; Sigma–Aldrich, USA); sodium acetate (anhydrous, ≥99.0%; Sigma–Aldrich, USA); sodium hydroxide (99.99%; Sigma–Aldrich, USA); [1,1′,2,2′^{2}H$_4$] dopamine hydrochloride (=DA·HCl-d_4, ≥98.0%; Cambridge Isotope Laboratories Inc., USA); 3-methoxytyramine hydrochloride (=3-MT·HCl, ≥99.0%; Fluka, Switzerland), water