Journal of Chromatography B, 998-999 (2015) 63–71

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Reassessment of the antioxidative mixture for the challenging electrochemical determination of dopamine, noradrenaline and serotonin in microdialysis samples

Jolien Van Schoors, Charlotte Lens, Katrien Maes, Yvette Michotte, Ilse Smolders, Ann Van Eeckhaut*

Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neurosciences, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Jette, Belgium

ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 18 May 2015
Accepted 12 June 2015
Available online 2 July 2015

Keywords:
In vivo microdialysis
Monoamine neurotransmitters
Stability
UHPLC
Electrochemical detection

ABSTRACT

In recent years, the simultaneous monitoring of the monoamine neurotransmitters dopamine, noradrenaline and serotonin in vivo is advancing due to innovations in miniaturized and fast chromatographic techniques. However, the determination of the most hydrophilic compound, noradrenaline, in microdialysis samples by (ultra-)high performance liquid chromatography ((U)HPLC) with electrochemical detection (ECD) is impeded by a broad solvent front, caused by the addition of antioxidative agents. Hence, an elaborate reassessment of currently used antioxidative mixtures is necessary for further analytical improvements. The proposed mixture, containing 100 mM acetic acid, 0.27 mM Na2EDTA and 12.5 μM ascorbic acid (pH 3.2), is less complex than previously described mixtures and shows minimal ECD interference. It stabilizes the three monoamines in standard solutions and in microdialysis samples, considering both autosampler stability at 4 °C for 48 h and long term stability at −20 °C for a duration of six months. An in vivo microdialysis experiment demonstrates the possibility to monitor changes in extracellular levels of the three monoamines simultaneously in the rat hippocampus with UHPLC–ECD using the optimized antioxidative mixture.

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1. Introduction

The monoaminergic system, covering the biogenic amines dopamine (DA), noradrenaline (NAD) and serotonin (5-HT), is an interesting pharmacological target for the treatment of neurological disorders such as Parkinson's disease [1–3], Alzheimer's disease [4,5], epilepsy [6–8] and neuropsychiatric disorders [7] and is therefore still subject of various innovative research projects. To quantify changes in the extracellular release of the monoamines in a specific brain area in response to a pharmacological stimulus, the in vivo microdialysis sampling technique is typically applied [9,10], combined with ion-pair (ultra-) high performance liquid chromatography ((U)HPLC) coupled to electrochemical detection (ECD) [9,11–16]. Thanks to the latest advances in miniaturized and high throughput techniques, researchers can start exploring more challenging, small brain areas which may contain particularly low extracellular monoamine levels (pM range). Such brain areas are often difficult to investigate because of the limited spatial resolution and require smaller microdialysis probes and highly precise stereotaxical implantation. Furthermore, current trends in neuropharmacology ask for high temporal resolution and multi-target strategies in order to catch fast and multiple neurotransmitter signals. This all asks for fast, selective and sensitive analytical techniques able to handle small sample volumes (μL range).

The limiting factor for the simultaneous (U)HPLC–ECD determination of the three monoamines in microdialysis samples is the low retention behavior of the most hydrophilic compound, NAD. The majority of the published analytical methods therefore only focuses on DA and 5-HT, whether or not including their respective metabolites. The addition of antioxidative agents to standard solutions and samples impedes the determination of the early eluting compound NAD because of a broadening of the injection solvent front on the chromatogram [14]. Hence, a reevaluation of the antioxidative mixture to ensure stability of the oxidizable monoamine structures is of importance.

Abbreviations: ECD, electrochemical detection; DA, dopamine; NAD, noradrenaline; 5-HT, serotonin; NET, noradrenaline transporter; SERT, serotonin transporter; DAT, dopamine transporter.

* Corresponding author at: Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neurosciences, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Jette, Belgium. Fax: +32 2 477 41 13.
E-mail address: aveeckha@vub.ac.be (A. Van Eeckhaut).

http://dx.doi.org/10.1016/j.jchromb.2015.06.010
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The catechols DA and NAD seem to be stable in acidic medium; however, they are oxidized to semiquinone and quinone products under neutral and alkaline conditions [17–20]. In contrast, indoleamines do not tolerate acidic conditions [21–23]. 5-HT is found stable in Ringer's solution at pH 6 yet unstable in acidified solutions [23–26]. Furthermore, instability of 5-HT in artificial cerebrospinal fluid but stability in microdialysis samples has been reported [27]. Nonetheless, considering simultaneous monoamine analysis, stability of both catecholamines and indolamines is required for samples kept in the autosampler prior to analysis as well as during long term storage, usually under freezing conditions. Different studies confirm the importance of reduced temperature and adequate sample pretreatment to limit monoamine oxidation [23,28]. Usually, microdialysis samples are acidified with acetic acid, perchloric acid or formic acid after collection. Subsequently, the samples are analyzed immediately or frozen at −20 °C or −80 °C prior to analysis [14,29–36]. Few researchers analyze the samples directly as such [28,37–39] or do not mention any precaution to ensure stability [16,40–43]. Others combine an acidification with the addition of antioxidative and/or chelating agents [2,7,12,20,25,44,45]. An antioxidative mixture developed in-house by Thorré et al. consists of 500 μM ascorbic acid and 3.3 mM L-cysteine as reducing agents, 0.27 mM Na_2EDTA as complexing agent, and 100 mM acetic acid to obtain a pH of 3.2 and provides excellent stability of DA, 5-HT and their metabolites in standard solutions for a duration of 20 h at 10 °C [20]. According to McKay et al. and Kankaanpää et al., the addition of ascorbic acid is the primary cause of the large solvent front in brain samples [25,46]. The latter author used an antioxidative mixture similar to that of Thorré et al. [20] and replaced ascorbic acid by oxalic acid in order to eliminate chromatographic interference for the determination of DA, 5-HT and their metabolites. To the best of our knowledge, no literature data are available regarding the stability of the monoamines, including NAD, for their simultaneous determination using (U)HPLC–ECD. Furthermore, stability studies for DA and 5-HT are often performed using standard solutions with concentrations irrelevant for microdialysis samples.

The aim of the present work is to reassess the antioxidative mixture proposed by Thorré et al. [20] for preservation of the three monoamines DA, NAD and 5-HT in microdialysis samples with minimal chromatographic interference in order to shift the frontiers for further analytical improvements. Therefore, we studied the influence of a number of antioxidative agents on the size of the solvent front as well as on the stability of the monoamines in standard solutions and in microdialysis samples for a limited period of time in the autosampler and for a long term in the freezer. Subsequently, the performance of the described UHPLC–ECD assay is evaluated, including the examination of precision, accuracy, sensitivity, linearity and matrix effects. Ultimately, the suitability of the analytical method with use of the optimized antioxidative mixture for in vivo measurements is demonstrated by performing a microdialysis experiment during which the three monoamines are quantified simultaneously in response to an elevated potassium concentration and a pharmacological stimulus (imipramine and GBR 12909).

2. Material and methods

2.1. Chemicals and reagents

DL-noradrenaline hydrochloride, dopamine hydrochloride, serotonin hydrochloride, GBR 12909 dihydrochloride, imipramine hydrochloride, L-ascorbic acid, L-cysteine, citric acid monohydrate, phytic acid disodium salt, sodium acetate trihydrate, sodium chloride, sodium decanesulfonate and sodium hydroxide pellets are purchased from Sigma–Aldrich (Steinheim, Germany). Gallic acid, oxalic acid dihydrate, potassium chloride and sodium metabisulfite ($Na_2S_2O_5$) are supplied by Merck (Darmstadt, Germany). Calcium chloride hexahydrate and disodium edetate (Na_2EDTA) are purchased from Fluka (Steinheim, Germany). Diazepam 10 mg/2 mL is purchased as Valium® from Roche (Cenexi, Fontenay-sous-Bois, France), ketamine 1000 mg/10 mL as Ketamine 1000® from Ceva (Libourne, France), Ketoprofen 1% as Ketofen® from Merial (Toulouse, France) and NaCl 0.9% from Baxter (Lessines, Belgium). Purified water is obtained via an Arium® pro UV system of Sartorius Stedim Biotech (Vilvoorde, Belgium). Hydrochloric acid 37% is from Acros Organics (Geel, Belgium), acetonitrile HPLC grade and glacial acetic acid from Fisher Scientific (Loughborough, Leics, United Kingdom) and acetonitrile for ULC–MS from Biosolve (Valkenswaard, The Netherlands).

2.2. Preparation of standard solutions

2.2.1. Working solutions

Stock standard solutions of 1 mM DA, 1 mM NAD and 1 mM 5-HT are prepared in a solution containing 10 mM HCl, 5.26 mM $Na_2S_2O_5$ and 0.27 mM Na_2EDTA.

Standard working solutions (0.5 nM, 1.0 nM and 1.5 nM) of DA, NAD and 5-HT are prepared by dilution of the stock standard solutions in a mixture of four parts of a modified Ringer's solution (147 mM sodium chloride, 4 mM potassium chloride and 2.3 mM calcium chloride hexahydrate) and one part of a studied antioxidative mixture. The initial antioxidative mixture contains 100 mM acetic acid, 3.3 mM L-cysteine, 0.27 mM Na_2EDTA and 12.5 μM ascorbic acid, based on the in-house developed mixture of Thorré et al. [20], though with a decreased concentration of ascorbic acid in order to minimize the size of the solvent front on the chromatogram.

2.2.2. Calibration curves and quality control samples

Standard calibration curves (100 pM, 250 pM, 500 pM, 750 pM, 1000 pM, 1250 pM and 1500 pM) are prepared by dilution of the stock standard solutions in a mixture of four parts of a modified Ringer's solution (147 mM sodium chloride, 4 mM potassium chloride and 2.3 mM calcium chloride hexahydrate) and one part of the optimized antioxidative mixture (100 mM acetic acid, 0.27 mM Na_2EDTA and 12.5 μM ascorbic acid).

Quality control samples are prepared by spiking of pooled basal microdialysates, originating from the hippocampus of three rats. The final concentrations are the same as these of the standard solutions in the calibration curve and the solvent is composed of four parts of microdialysate and one part of the optimized antioxidative mixture.

2.3. In vivo microdialysis experiment

In vivo experiments are carried out according to the national guidelines for animal experiments with the permission of the local ethical committee of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel (VUB).

Male albino Wistar rats (250–300 g) purchased from Charles River Laboratories (Cedex, France) are used. The rats are anaesthetized prior to surgery with a ketamine/diazepam mixture (60/4.5 mg/kg) i.p. and received ketoprofen (3 mg/kg) s.c. An intracerebral guide cannula is stereotaxically implanted in the hippocampus of both hemispheres (AP: −5.6, L: ± 4.6, V: + 4.1 relative to bregma, according to the atlas of Paxinos and Watson [47]) and fixed with dental cement. After recovery, a microdialysis probe (MAB 6.14.3, 0.6 mm outer diameter, 3 mm length, 15 kDa cut-off) of Microbiotech (Stockholm, Sweden) is inserted into each cannula. The probes are continuously perfused with modified Ringer's

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