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Recent contributions of capillary electrophoresis to neuroscience

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ABSTRACT

Contributions to neuroscience are necessary to understand the behavior of the brain. Powerful analytical techniques are needed to monitor neuroactive molecules and their concentrations in biological samples (fluids, cells, and brain tissues). Capillary electrophoresis (CE) is well known for its high resolution power, short analysis times, and low consumption of reagents and samples. It presents analytical advantages for the determination of neuroactive molecules not easily determined by other analytical techniques. CE also offers the possibility of controlling more than one neuroactive molecule at a time, making it interesting to detect changes as a result of a stimulus. CE is well established to accomplish enantioseparations, contributing a better understanding of the properties of a neuroactive chiral molecule. This review focuses on the most relevant articles published from January 2008 to July 2014, based on the determination in biological samples of potentially interesting molecules in neuroscience using CE and microchip-CE.

Contents

Abbreviations: 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, Serotonin; μ-TAS, Micro-total analysis system; ABEI, N-(4-aminobutyl)-N-ethyl isoluminol; ACh, Acetylcholine; AD, Amperometric detection; Ala, Alanine; Arg, Arginine; Asn, Asparagine; Asp, Aspartate; BGE, Background electrolyte; BPA, Bisphenol A; Br-BQCA, 3-(4-bromobenzoyl)-2-quinolinecarboxaldehyde; CD, Cyclodextrin; CEC, Capillary electro-chromatography; C^4D, Capacitive coupled contactless conductivity detection; CE, Capillary electrophoresis; CFSE, 5-carboxyfluorescein N-succinimidyl ester; Cit, Citruline; CL, Chemiluminescence; CNS, Central nervous system; CSF, Cerebrospinal fluid; CSP, Chiral stationary phase; Cys, Cysteine; CZE, Capillary-zone electrophoresis; DA, Dopamine; DM-β-CD, Dimethyl-β-CD; DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylaceticacid; DTAF, 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein; EC, Electrochemical; EKC, Electrokinetic chromatography; EP, Epinephrine; FASS, Field-amplified sample stacking; FITC, Fluorescein isothiocyanate; FSCV, Fast-scan cyclic voltammetry; GABA, γ-aminobutyric acid; Gln, Glutamine; Glu, Glutamate; Gly, Glycine; Him, Histamine; His, Histidine; HPA-β-CD, 6-monodeoxy-6-mono(3-hydroxy)-propylamino-β-cyclodextrin; HPLC, High-performance liquid chromatography; HP-β-CD, hydroxypropyl-β-CD; HVA, Homovanillic acid; Ile, Isoleucine; IT, Ion trap; IXS, 3-indoxyl sulfate; LED, Light-emitting diode; Leu, Leucine; LIF, Laser-induced fluorescence; LINF, Laser-induced native fluorescence; LVSS, Large-volume sample stacking; Lys, Lysine; MCE, Microchip capillary electrophoresis; Met, Methionine; MEKC, Micellar electrokinetic chromatography; MEEKC, Micro-emulsion electrokinetic chromatography; MIP, Molecularly-imprinted polymer; MISPE, Molecularly-imprinted solid-phase extraction; MMIP, Magnetic molecularly-imprinted polymer; NBD-F, 7-nitrobenzo-2-oxa-1,3-diazole; NDA, Naphthalene-2,3-dicarboxyaldehyde; NE, Norepinephrine; NM, Normetanephrine; OP, Octopamine; OPA, *o*-phthalaldehyde; Orn, Ornithine; PBS, Phosphate buffer saline; PDDAC, Poly(diallyldimethylammonium) chloride; PDMS, Polydimethylsiloxane; Phe, Phenylalanine; Pro, Proline; Q, Quadrupole; SDS, Sodium dodecyl sulfate; Ser, Serine; SPE, Solid-phase extraction; SPME, Solid-phase microextraction; TA, Tryptamine; Tau, Taurine; TCA, Trichloroacetic acid; Thr, Threonine; TOF, Time-of-flight; Trp, Tryptophan; Tym, Tyramine; Tyr, Tyrosine; Val, Valine; VMA, Vanillomandelic acid.

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1. Introduction

In neuroscience, the study of the nervous system, neurons play the most important role, since they are the cells in charge of transmitting information by electrical and chemical signals. Signal transmission can be driven by chemical messengers, also known as neuroactive compounds (neurotransmitters or neuromodulators), which are involved in the signal transmission occurring in the synapse of the neuron. In this synapse, the neuroactive compounds are released to bind to the receptors in the membrane of a target cell [1]. Neurotransmission has proved to be related to the behavioral, cognitive, and emotional state of an organism and also to be associated with certain conditions, such as depression, drug dependence, schizophrenia, and degenerative diseases [2]. Most diseases of the central nervous system (CNS) are caused and can be accentuated by complex and abnormal disturbances or disruptions of regulatory mechanisms, protein-expression profiles or some metabolic pathways [3].

A large variety of molecules can be neurologically active, ranging from gases, such as nitric oxide, carbon monoxide and hydrogen sulfide, to small molecules, such as amino acids (both protein and non-protein) and biogenic amines (monoamines, histamine and acetylcholine), and larger molecules, such as neuropeptides or hormones [1,4]. Glycine (Gly), taurine (Tau) and γ-aminobutyric acid (GABA) are the main inhibitory amino acids [2], in contrast to glutamate (Glu) and aspartate (Asp), which are the most widespread excitatory neuroactive compounds in the CNS and influence numerous neuronal networks [1]. Serotonin (5-HT), a monoamine derived from tryptophan (Trp), is implicated in physiological functions, such as memory, learning, feeding, sleep and body-temperature regulation and it is also involved in pathologies, such as depression, Alzheimer's disease, autism, schizophrenia, and bipolar disorder [1]. Other monoamines, such as catecholamines dopamine (DA), epinephrine (EP), and norepinephrine (NE), all derived from tyrosine (Tyr) and phenylalanine (Phe), are also important molecules in neurotransmission. These catecholamines play an important role in the diagnosis of many disorders, such as Alzheimer's [5] and Parkinson's diseases [6], cocaine addiction, pheochromocytoma and a variety of mental diseases [7]. Other important neuroactive molecules are: the biogenic amine histamine (Him), which is involved in a large variety of physiological responses (regulation of sleep, secretion of hormones, and formation of cognition) [8,9]; and, acetylcholine (ACh), which was the first neuroactive molecule discovered and it is known that dysfunction in the cholinergic system is related to Alzheimer's and Parkinson's diseases [1].

The potential role of D-amino acids in aging and neurodegenerative processes, such as Alzheimer's disease, was revealed years ago by Fisher and D'Aniello [10,11] and this role has been studied ever since [12]. D-serine (D-Ser), D-alanine (D-Ala), and D-Asp have all been found in relatively significant levels in the CNS [13]. D-Ser plays an important role in neuroplasticity, memory, and learning [12], and the amounts of D-Ser and D-Ala in mammals have been related to schizophrenia and depression [13,14]. D-Asp has been related to some neuromodulatory functions and it is involved in developmental and endocrine functions [12]. In the case of catecholamines, it is known that interaction of L/D-EP and L/D-NE with their receptors is stereoselective, with their enantiomers exhibiting different activity and selectivity [15], although no clear relation between neurotransmission function and enantiomers has been established yet.

The above information points out the importance of the development of analytical tools to determine these small molecules to study neurological disorders, to measure and to evaluate the progress of a disease or a process that occurs in the CNS, and to find the possible response to a specific treatment [3]. Also, multiple analyte detection is attractive from the point of view of neuroscience because the alteration of more than one neuroactive substance at a time can be studied in response to a stimulus. Thus, analytical techniques frequently employed for monitoring neuroactive molecules lead to rapid, sensitive, and efficient analysis, and should offer the possibility of performing it in samples of significantly low volumes (some of them just few μL) and at very low concentrations of the compounds of interest (sometimes even below the nM level).

Some review articles [4,16] have focused on the application of different analytical techniques for determination of neuroactive compounds, with high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), enzyme assays and biosensor microelectrodes being the most widely used. Among all these techniques, CE can be considered a suitable and a reliable technique to study potentially interesting molecules in neuroscience because of its high resolution power, fast analysis and its use of small sample volumes (nL or even less) what makes it ideal for *in-vitro* or *in-vivo* analysis of neurological samples.

Moreover, chiral analysis represents a promising topic in determination of neuroactive compounds because of the differences in the biological activities of a pair of enantiomers. An analytical technique capable of resolving enantiomers should therefore facilitate understanding of the activity of the enantiomeric neuroactive compounds. In this aspect, CE is considered as one of the most powerful and useful techniques in chiral separations due to its wide possibilities [17,18].

Contributions of CE to neuroscience have been summarized in different review articles [2,19]. Taking into account that different works have been published since, our aim is to cover recent articles, published from January 2008 to July 2014, taking the review published in 2008 [2] as the starting point. We consider all relevant articles published within the period of time mentioned and focusing on the determination by CE and microchip CE of neuroactive compounds in biological samples of interest in neuroscience. Our work sorts the CE methods by the detector system employed, highlighting the differences among them and indicating the instrumentation needed. As a consequence, methods with different detection systems can be easily followed and compared. We also discuss the importance and the possibilities for sample preparation, preconcentration techniques, and chirality in determination of potentially interesting molecules in neuroscience, whose contributions have been increasing since 2008.

2. Capillary electrophoresis for determination of neuroactive compounds

2.1. Sample preparation

Analysis of biological samples provides very useful information when studying neuroactive molecules of interest in neuroscience research. The most widely analyzed samples are different biological fluids, cells, and brain tissues. Biological fluids, such as urine, blood (plasma, serum, and whole blood), cerebrospinal fluid (CSF), and extracellular fluid (ECF) of certain regions of the brain, are the fluids analyzed to determine neuroactive molecules, e.g.:

- determination of catecholamines in urine is a well-known tool in the diagnosis of pheochromocytoma [20];
- plasma levels of neuroactive amino acids can be used in the diagnosis of several disorders, such as bipolar disorder [21];
- CSF is very useful in the diagnosis of Alzheimer's disease [22]; and,
- ECF can be useful to study the effects in the brain during ischemia and reperfusion periods [23].

The analysis of single mammalian cells, such as PC-12 nerve cells [24], or nerve cells from invertebrates, such as the sea slug *Aplysia californica* [25], is carried out to increase knowledge regarding CNS behavior.