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Advances and challenges in neurochemical profiling of biological samples using mass spectrometry coupled with separation methods

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ABSTRACT

Neurochemicals are mainly distributed in the central nervous system, and related to various neurological functions and disorders. Because neurochemicals are biosynthesized and metabolized through multiple pathways, it is difficult to understand the overall nervous system or to diagnose neurological disorders based on certain neurochemical levels. Therefore, to learn more about the nervous system and neurological disorders, metabolomics approaches should be used to profile the metabolic pathways of neurochemicals. To date, the neurochemical profiling has been pursued by various methods, especially including mass spectrometry (MS) coupled with conventional chromatographic techniques, which can sensitively and selectively determine numerous neurochemicals in biological samples. Nevertheless, the development of chromatographic MS-based analytical platforms to profile neurochemicals in biological samples has faced several challenges. This review was organized to help characterize neurochemicals using descriptions of the basic procedures (sample preparation and chromatographic MS detection). Moreover, we describe clinical applications and performance evaluation of these techniques.

1. Introduction

As important components of the nervous system, diverse neurochemicals (neurotransmitters, such as acetylcholine, gamma-amino butyric acid, and glutamate; neuromodulators, such as dopamine, norepinephrine, epinephrine, and serotonin; and their precursors and metabolites) significantly affect the interactions between neurons and other cells (Fig. 1 and Table 1S) [1,2]. For example, neurochemicals can affect brain functions because behavioral, cognitive, and emotional conditions are all encoded by neurochemical interactions between neurons [3]. They can also participate in various cellular functions, such as proliferation, maturation, and apoptosis [4,5]. The various forms of neurochemicals present in cells, tissues, and body fluids are the subcategory of the *metabolome* of which levels are affected by the concentrations and activities of biosynthetic and metabolic enzymes and by changes in environmental variables (diet, drug, and lifestyle) [6,7]. Because metabolic homeostasis regulates the integrity of the whole body, abnormalities in neurochemical pathways might be closely correlated with (or be tightly reflected by) numerous neurological disorders such as Alzheimer's disease, Parkinson's disease, major depressive disorder, and schizophrenia (Fig. 2) [8].

It is well perceived that mental and physical health cannot be properly maintained in the presence of neurological disorders. Patients with neurological diseases have generally been deprived of balanced social interactions and are often subjected to economic destitution. In other words, neurodegenerative disorders have socioeconomic costs [9]. To help resolve those problems, it is important to secure proper methods for the accurate diagnosis and effective treatment of such diseases, but at this time, few relevant objective indices exist. Therefore, it is necessary to develop diverse approaches, including metabolomics analyses, to better understand the pathogenic mechanisms involved [10–12].

2. Sample preparation

2.1. Storage of biological matrices

As neurochemicals are widely distributed in the human body by the circulatory system, they are also found ubiquitously from

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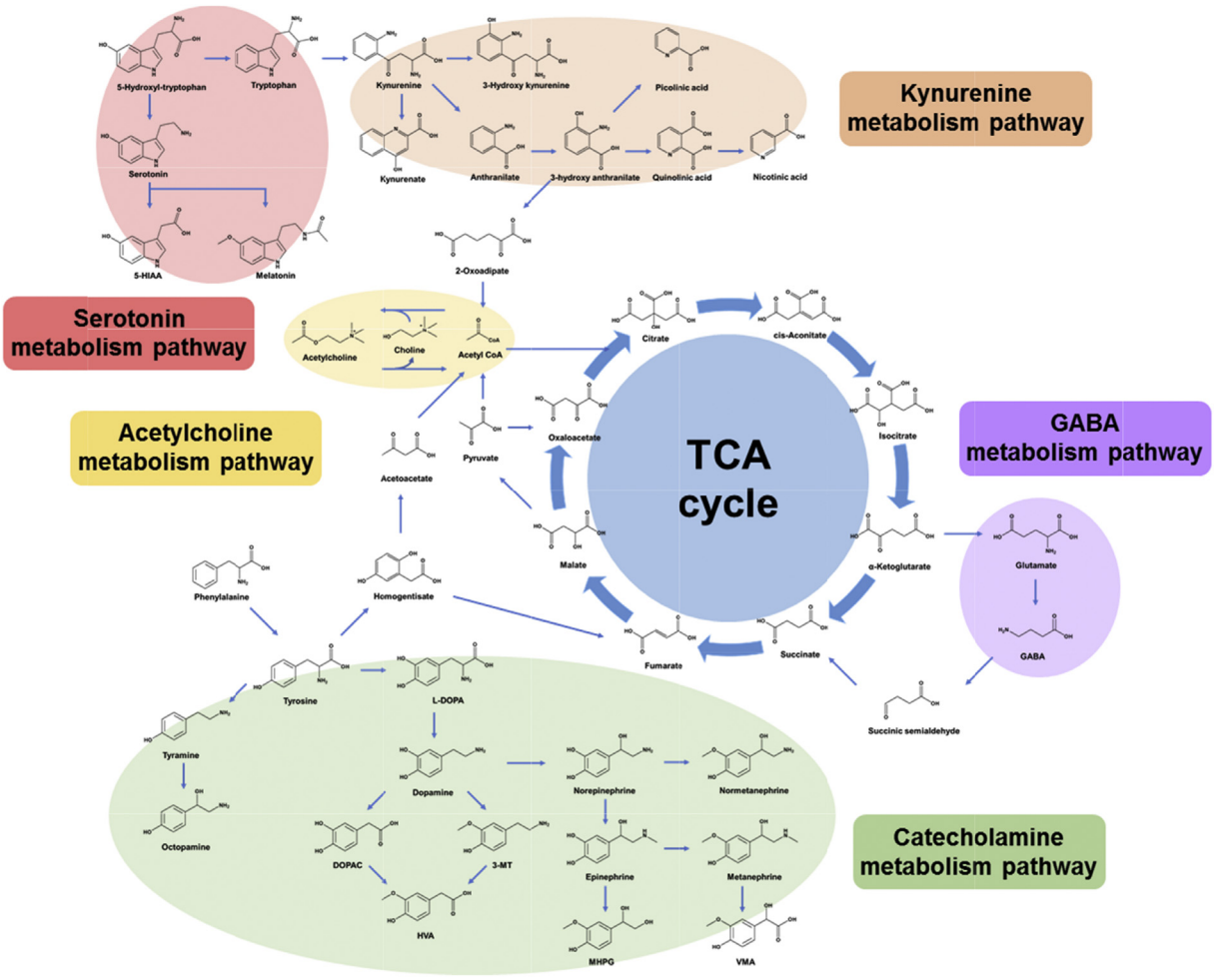

Fig. 1. Major metabolism pathways for neurochemicals in the human body.

various biological samples. As neurochemicals exist everywhere in the body system, various biological samples collected through invasive/non-invasive methods can apply to multiple neurochemical research (Fig. 3). After collection from subjects, biological samples can be subject to residual enzymatic activity that can significantly alter the neurochemical profile [13]. Therefore, those enzymes must be eliminated or their activities suppressed. In addition, specific classes of neurochemicals (such as catecholamines) are vulnerable to environmental conditions (e.g., temperature, sample pH, and storage time) [14]. Thus, it is critical to understand and optimize the conditions for collecting and storing samples to accurately quantify intact neurochemicals.

One of the easiest ways to quench enzymatic activity and the degradation of neurochemicals is to freeze biological samples (e.g., urine and blood) upon collection. Urine can be stored in an otherwise unpreserved form at −80 °C for 22 days without any significant fluctuation in neurochemicals (variations within 10%) [15]. Acidified urine samples (below pH 3.5) were stable for several months at −20 °C [16]. A consensual protocol for blood samples calls for centrifugation within 1 h of collection to make plasma [14]. Neurochemicals in plasma are stable at −20 °C for 6 weeks with sodium metabisulfite [17], and they are stable at −80 °C without any antioxidants for more than 6 years [18]. Thus, for long-term storage of urine samples, preserving them at −80 °C with acidification is recommended. Likewise, for plasma samples, −80 °C plus antioxidants is a preferable option for storage, because the catechol group of catecholamines (such as dopamine (DA), epinephrine (E), and norepinephrine (NE)) is prone to oxidation under neutral or alkaline conditions [14,16,19].

2.2. Extraction and cleanup

To effectively eliminate matrix interference and selectively extract neurochemicals, many researchers have developed procedures for sample preparation. For instance, biological fluids such as urine and plasma have been diluted with water [20] and centrifuged for protein precipitation [21,22]. Also, solid phase extraction (SPE), initially developed to extract analytes of interest from complex matrices, has been applied to biological samples such as human urine [23,24], plasma [25], and peripheral blood mononuclear cells [26]. Likewise, solid phase microextraction (SPME) has been used to simultaneously preconcentrate and extract neurochemicals in human urine [27,28]. Miniaturized SPE techniques, such as microextraction in a packed syringe (MEPS) and pipette tip SPE (PT-SPE), have been developed to extract neurochemicals from human and mouse urine, respectively [29,30]. MEPS and PT-SPE use a gas-tight syringe and pipette tips filled with a small volume of SPE sorbents, respectively (Fig. 1S (A) and 1S (B)). The miniaturization of SPE tools might make it possible to achieve a further reduction in sample size, solvent quantity, and extraction time.

In recent years, liquid-liquid extraction (LLE) using ethyl acetate as the water-immiscible extraction solvent was developed to