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Simultaneous quantification of neuroactive dopamine serotonin and kynurenine pathway metabolites in gender-specific youth urine by ultra performance liquid chromatography tandem high resolution mass spectrometry

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ABSTRACT

Neuroactive metabolites in dopamine, serotonin and kynurenine metabolic pathways play key roles in several physiological processes and their imbalances have been implicated in the pathophysiology of a wide range of disorders. The association of these metabolites' alterations with various pathologies has raised interest in analytical methods for accurate quantification in biological fluids. However, simultaneous measurement of various neuroactive metabolites represents great challenges due to their trace level, high polarity and instability. In this study, an analytical method was developed and validated for accurately quantifying 12 neuroactive metabolites covering three metabolic pathways in youth urine by ultra performance liquid chromatography coupled to electrospray tandem high resolution mass spectrometry (UPLC-ESI-HRMS/MS). The strategy of dansyl chloride derivatization followed by solid phase extraction on C18 cartridges were employed to reduce matrix interference and improve the extraction efficiency. The reverse phase chromatographic separation was achieved with a gradient elution program in 20 min. The high resolution mass spectrometer (Q Exactive) was employed, with confirmation and quantification by Target-MS/MS scan mode. Youth urine samples collected from 100 healthy volunteers (Female:Male = 1:1) were analyzed to explore the differences in metabolite profile and their turnover between genders. The results demonstrated that the UPLC-ESI-HRMS/MS method is sensitive and robust, suitable for monitoring a large panel of metabolites and for discovering new biomarkers in the medical fields.

1. Introduction

Neurotransmitters and their metabolites are widely distributed in the central and peripheral nervous systems, playing significant roles in maintaining various physiological functions. Their alternations are closely associated with many neurological disorders, such as Alzheimer's disease [1], schizophrenia [2], anxiety [3], depression [4] and Parkinson's disease [5]. Fig. 1 shows the structures of the main neuroactive metabolites spanning dopamine and tryptophan pathways examined in this study. All these compounds are derived from aromatic amino acids like tryptophan and tyrosine by the action of aromatic amino acid decarboxylase enzymes. Previous studies have demonstrated that information on the rates of some specific monoamine production, metabolism and excretion were useful for psychological studies [6]. However, in contrast to the studies that focused on the changes of some specific neurotransmitters with disorders, such as dopamine and serotonin, more recent studies have insights in metabolic pathways to explore the overall balance of the neuropsychological network system. The dopamine and serotonin metabolic pathways have been a topic of intense research given its well-known action as neurotransmitters [6]. Meanwhile, the kynurenine pathway, another branch

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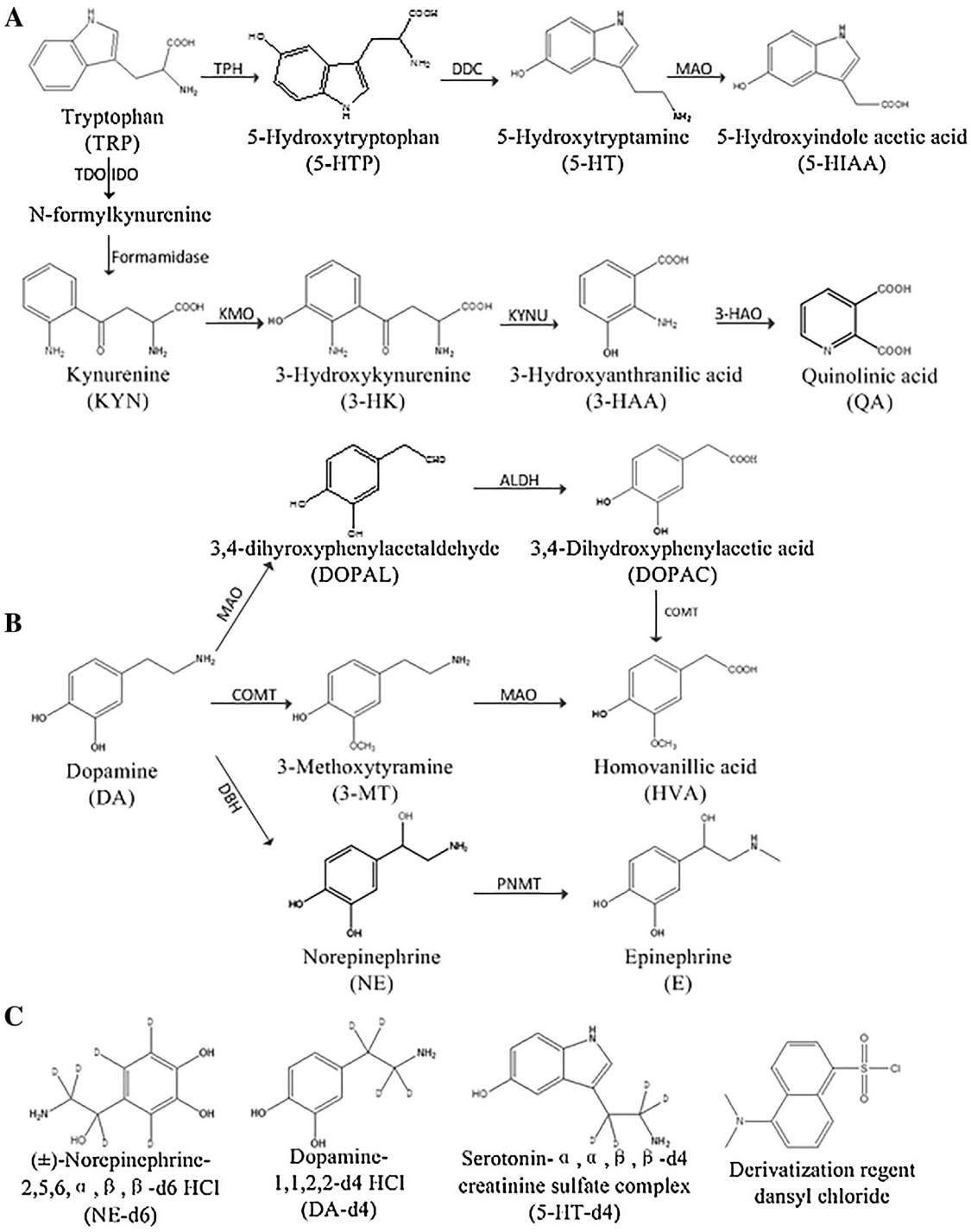

Fig. 1. The major metabolites investigated in this study: (A) Serotonin and kynurenine branches on the metabolic pathway of tryptophan. (B) Dopamine metabolic pathway. (C) Internal standards and the derivatization reagent.

of tryptophan metabolism aside from the serotonin route, has received much interest in this decade [7,8]. The metabolism of tryptophan to kynurenine is facilitated by the enzyme indoleamine 2,3-dioxygenase (IDO) and it generates a series of neuroactive compounds, the so called kynurenines, such as 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA). Currently, 3-HK and 3-HAA are mainly being studied, since they have shown to induce neurotoxic effects by suppressing T cell responses via intracellular reactive oxygen species formation or glutathione depletion [9,10] or activating glutamate receptors to influence a large group of functions in the central nervous system [11].

Due to these neuroactive metabolites are of great clinical importance in the diagnosis of several diseases, a global profiling of the precursors and multiple downstream metabolites will provide valuable information for the disease pathology and drug intervention. To this end, it is highly desirable to develop an efficient approach for simultaneous determination of these compounds in biological fluids, such as brain tissue, plasma, microdialysate and urine [12–14]. Particularly, the analysis of urine samples