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Determination of catecholamines in plasma and urine

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For more than 20 years, measurement of catecholamines in plasma and urine in clinical chemistry laboratories has been the cornerstone of the diagnosis of neuroendocrine tumors deriving from the neural crest such as pheochromocytoma (PHEO) and neuroblastoma (NB), and is still used to assess sympathetic stress function in man and animals. Although assay of catecholamines in urine are still considered the biochemical standard for the diagnosis of NB, they have been progressively abandoned for excluding/confirming PHEOs to the advantage of metanephrines (MNs). Nevertheless, catecholamine determinations are still of interest to improve the biochemical diagnosis of PHEO in difficult cases that usually require a clonidine-suppression test, or to establish whether a patient with PHEO secretes high concentrations of catecholamines in addition to metanephrines.

The aim of this chapter is to provide an update about the catecholamine assays in plasma and urine and to show the most common pre-analytical and analytical pitfalls associated with their determination.

Catecholamine biosynthesis

Catecholamines result from multi step biotransformation of tyrosine by a cascade of enzymes in neuronal structures of the central and peripheral nervous system. In circulation, the most abundant

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catecholamines are norepinephrine (NE), epinephrine (E) and dopamine (DA). E and NE are produced by the adrenomedullary chromaffin cells whereas NE is also synthesized by sympathetic nerve endings. DA essentially results from the decarboxylation of 3,4-dihydroxyphenylalanine (L-DOPA) provided by the diet. Catecholamine synthesis is initiated by tyrosine hydroxylase (TH) generating L-DOPA from tyrosine. Conversion of tyrosine to L-DOPA by TH is the rate-limiting step in catecholamine biosynthesis. L-DOPA is then decarboxylated to dopamine via aromatic L-amino acid decarboxylase, which is then converted to NE via dopamine beta-hydroxylase; and eventually to E by the action of phenylethanolamine N-methyltransferase (PNMT) in adrenomedullary cells [1].

Catecholamine metabolism

The inactivation and metabolism of catecholamines is beyond the scope of this review but it is crucial to keep in mind that only a small fraction of catecholamines released from storage vesicles of sympathetic nerves will enter into the bloodstream because of their neuronal reuptake through specific transporters followed by inward active transport into storage pools [1]. These pools present a constant dynamic equilibrium controlled by vesicular monoamine transporters (VMAT) leading to a leakage into the cytoplasm of catecholamines that are also available for oxidative deamination by monoamine oxidase (MAO) into 3,4-dihydroxyphenylglycol (DHPG). Additionally, catecholamines may enter non-neuronal tissues to be methoxylated into metanephrines (NE to normetanephrine [NMN] and E to metanephrines [MN]) by catechol-O-methyl transferase (COMT). In contrast to sympathetic neurons, the adrenal medulla chromaffin cells express COMT and may also produce MNs. However, extraneuronal O-methylation of NE and E to MNs represents minor pathways of metabolism compared to intraneuronal deamination. Oxidation of MN and NMN by MAO or methoxylation of DHPG by COMT leads to the formation of methoxyhydroxyphenylglycol (MHPG) that is further metabolized in the liver into vanyllilmandelic acid (VMA). These mechanisms explain in part the short half-life of plasma catecholamines. The dietary source of catecholamines undergoes a sulfoconjugation by a specific sulfotransferase isoenzyme SULT1A3 in the gastrointestinal tract before reaching the systemic circulation. Thus, free catecholamine concentrations that are measured in plasma and urine are not influenced by feeding. Consequently, 99% of dopamine and some 60–70% of circulating NE and E are sulfate-conjugated [1]. Unconjugated dopamine in urine is tenfold more abundant than NE, since it is derived from renal extraction and decarboxylation of circulating 3,4-dihydroxyphenylalanine (L-DOPA) provided by food, and may potentially be a misleading parameter for PHEO detection [2]. The metabolism of dopamine shows similar extraneuronal metabolism by COMT and MAO leading to the formation of 3-methoxytyramine (MT) and 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC undergoes O-methy lation to produce homovanillic acid (HVA) as an end-product.

Measurement of catecholamines in pheochromocytoma workup

Analytical procedures and analytical pitfalls

The analysis of catecholamines has produced an extensive literature in terms of analytical methodology. In 1949, von Euler and Hamberg reported colorimetric assays that allowed differentiation of NE from E based on alumina oxide extraction [3]. Since these assays lack sensitivity they were replaced by fluorimetric assays that suffer from low specificity [4]. A major advance was done with radiometric-enzymatic assay for measuring simultaneously femtomole quantities of E, NE and DA, but this method is tedious [5]. High-performance liquid chromatography (HPLC) with fluorescence detection was a method of choice since catecholamines exhibited native fluorescence. Unfortunately, fluorescence detection suffers from major drawbacks since the emission wavelength for catecholamines is short and even after derivatization with chemical agents, fluorescence detection may be disturbed by endogenous co-eluting compounds [6]. Alpha-methyldopa and labetalol are classical sources of interferences observed when fluorescence detection is used [7,8]. In the early 80s, electrochemical detection (ECD) became popular since it allowed quantitating catecholamines in plasma from patients [9,10]. However, interference of labetalol causes spuriously raised E levels [11]. Paracetamol (acetaminophen) is also a cause of serious interferences with ECD methods requiring careful inspection of chromatograms to

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