Determination of catecholamines and their metabolites in urine by High Performance Liquid Chromatography for the diagnosis of pheochromocytoma and neuroblastoma

Pheochromocytoma and neuroblastoma are characterised by an enhanced production of catecholamines and their metabolites in the respective tissue, resulting in increased excretion of catecholamines in the urine. The urinary and plasma concentrations of catecholamines and their metabolites may then be several times higher than upper reference limits (1).

Catecholamines are a group of biogenic amines, adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine, as well as their derivatives. Their degradation products in urine are homovanillic acid (HVA) for dopamine and vanillylmandelic acid (VMA) for adrenaline and noradrenaline, all having diagnostic significance. Metanephrines are the inactive metabolites of epinephrine and norepinephrine; they have no hormonal effect, but do also have diagnostic value.

In normal circumstances catecholamines cause physiological changes that prepare the human body for physical activity (e.g. increases in glucose levels, blood pressure, heart rate etc.). Hence, in emergency and shock therapy, catecholamines are used as drugs (adrenaline, noradrenaline, dobutamine), mainly for the acute elevation of blood pressure.
In the organism catecholamines are synthesised from the aromatic amino acid tyrosine and play a central role as hormones and neurotransmitters (2). They are produced in the adrenal glands and in the nervous system. In the synthesis chain, initially, dopamine is produced from tyrosine (with DOPA as an intermediate). In turn, norepinephrine and epinephrine are derived from further metabolic modification of dopamine (fig. 1).

Catecholamines have a half-life of a few minutes when circulating in the blood. The inactivation by degradation of all catecholamines is regulated by the enzyme monoamine oxidase (MAO). Excretion of catecholamines released from the adrenal medulla and the nervous system is about 1% renally. 80-85% of the catecholamines are excreted as VMA, and approximately 15% as metanephrines. Adrenal gland-released hormones particularly must be inactivated enzymatically.

Of clinical importance in the diagnosis of pheochromocytoma and other tumours of the nervous system, is the determination of catecholamines and/or their metabolites in urine, plasma and tissue (3-6). A pheochromocytoma is a neuroendocrine tumour of the adrenal gland medulla (in the chromaffin cells), or of the extra-adrenal chromaffin tissue that failed to involute, and secretes large amounts of catecholamines. Pheochromocytoma is characterised by an overproduction of adrenaline, noradrenaline and dopamine, which is expressed by high blood pressure, sweating, headache and palpitations (tachycardia). Further signs are pale skin, blood sugar (hyperglycemia) level, leukocytosis, and weight loss. Significantly raised levels of adrenaline, noradrenaline or dopamine are a clear marker for the presence of a pheochromocytoma. About 10% of pheochromocytomas are malignant. However, if a pheochromocytoma is found in time, it is potentially curable.

Care should be taken to rule out other causes of increased catecholamine levels, such as hypoglycemia, stress, and drugs (nose and cough drops, bronchodilators (theophylline), diet pills, tetracyclines, methyldopa, α1- and β-antagonists) affecting catecholamine production. Various food (e.g. vanilla) can also influence the levels of urinary metanephrine and VMA. In contrast, catecholamine levels decrease when taking tricyclic antidepressants (e.g. reserpine) or alpha-2-agonists.

A second catecholamine-overproducing disease is neuroblastoma, which is a neoplastic disease of infants and
early childhood. These tumours also overproduce the acidic and methoxylated metabolites VMA, HVA and meta-nephrine. Examination of 24-hour urine collection for VMA (7), dopamine and its metabolites HVA provide about 80% of patients a definite diagnosis of a neuroblastoma. For infants it is recommended to use spontaneous urine samples related to creatinine values and to perform the analysis several times.

Screening for catecholamine-secreting tumours is preferably done by the quantitative determination of the 24 h excretion of catecholamines in urine. For tumour localisation or in pharmacological functional tests it is necessary to measure catecholamine concentration in plasma (1, 4-6, 8).

For patients with a family history of pheochromocytomas or neuroblastomas, routine screening of catecholamines should be considered.

In addition, the quantitative determination of urinary and plasma catecholamines is not only useful in the diagnosis of hypertension but also for the evaluation of a number of other clinical and pharmacological states. For example, the concentrations of noradrenaline and adrenaline are indicative of the activity of the sympathetic nervous system (9, 10), and are important parameters in congestive cardiac insufficiency, coronary heart disease, diabetes mellitus, arteriosclerosis, acute asthma, and others (11-19). Also, in scientific studies in areas such as stress research and sports medicine, (e.g. doping), catecholamine levels provide very useful information (20-23).

**Principle of Chromsystems Reagent kits**

For catecholamine analysis in urine high pressure liquid chromatography (HPLC) with electrochemical detection has offered a fast and reliable analysis method. For sample collection it is recommended to investigate an acidified 24-hour urine, as catecholamine excretion is subjected to strong circadian variations.

Chromsystems Instruments & Chemicals offers three different reagent kits for the HPLC analysis of Catecholamines and their metabolites in urine.

1) Reagent kit for HPLC analysis of Catecholamines in urine (Order no. 6000)
2) Reagent kit for HPLC analysis of Metanephrines in urine (Order no. 2020)
3) Reagent kit for HPLC analysis of VMA, HVA, 5-HIAA in urine (Order no. 1000/B)

These kits allow for the routine analysis of catecholamines, their metabolites and derivates in urine with an isocratic HPLC system and electrochemical detector. These molecules are extracted from the urine matrix with an ion exchange column for HPLC analysis. As no exact pH-adjustment of the urine samples for the ion exchange pre-column is necessary, the sample preparation is safe and easy (fig. 2). Moreover, sample preparation requires only two washing steps with water. A specific HPLC column in combination with a mobile phase optimised for this particular separation, allows sure and reliable chromatographic quantification and guarantees reproducible results. One person can analyse up to 100 urine samples per day.

Chromsystems is developing a method for the combined analysis of catecholamines and metanephrines (normetanephrine, meta-nephrine and 3-methoxytyramine) in order to determine these molecules with the same column and mobile phase.

Chromsystems Instruments & Chemicals also provide a reagent kit for HPLC to determine the catecholamines in plasma.

- Reagent kit for HPLC analysis of Catecholamines in plasma (Order no. 5000)
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Literature

5. Bravo EL. The Clinical Value of Catecholamine Measurement, Laboratory Management (June 1982).