



Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev



Review

Neurotransmitters excreted in the urine as biomarkers of nervous system activity: Validity and clinical applicability

David T. Marc*, Joseph W. Ailts, Danielle C. Ailts Campeau, Michael J. Bull, Kelly L. Olson

NeuroScience, Inc. 373 280th Street, Osceola, WI 54020, United States

ARTICLE INFO

Article history:

Received 7 June 2010
Received in revised form 21 July 2010
Accepted 30 July 2010

Keywords:

Neurotransmitter
Urinary
Nervous system
Biomarker
Depression
Inflammation
ADHD
Psychiatric

ABSTRACT

Strategies for managing the nervous system are numerous while methods of evaluating the nervous system are limited. Given the physiological importance of neurotransmitters as signaling molecules in the nervous system, the measurement of neurotransmitters has significant potential as a clinical tool. Of all the biological fluids that can be utilized, urinary neurotransmitter testing, due to its stability, sensitivity, and non-invasiveness, is the desired method to analyze nervous system function. Increasing use of this technology in a clinical setting demands a review of its feasibility, utility, and clinical value. We review the current body of literature pertaining to the mechanism of neurotransmitter transport across the blood–brain barrier as well as neurotransmitter filtration and excretion by the kidneys. In addition, this review summarizes the historical use of urinary neurotransmitter assessment to diagnose pheochromocytoma. Early research also correlated urinary assessment of neurotransmitters to various clinical symptoms and treatments of which we present research only for depression, ADHD, and inflammation because of the abundant amount of research in these areas. Finally, we review the limitations and challenges of urinary neurotransmitter testing. Taken together, evidence suggests that neurotransmitters excreted in the urine may have a place in clinical practice as a biomarker of nervous system function to effectively assess disturbances and monitor treatment efficacy.

© 2010 Elsevier Ltd. All rights reserved.

Contents

1. Introduction	00
1.1. History of urinary neurotransmitter measurement	00
1.2. Neurotransmitter transport and the blood–brain barrier	00
1.3. Neurotransmitter transport and the kidneys	00
1.4. Urinary neurotransmitters and CNS associations	00
1.5. Urinary neurotransmitters and depressive symptoms	00
1.6. Urinary neurotransmitters and ADHD	00
1.7. Urinary neurotransmitters and inflammation	00
1.8. Limitations of urinary neurotransmitter assessment	00
2. Conclusion	00
Acknowledgments	00
References	00

1. Introduction

Neurotransmitters are the primary chemical messengers released from neurons and relay, amplify and modulate signals to other cells. Due to the significant contribution of neurotransmitters to not only neurological functioning, but also endocrinological and

immunological actions, clinicians and researchers are interested in the function and measurement of neurotransmitters as they have the potential to serve as clinically relevant biomarkers for specific disease states or to monitor treatment efficacy (Cook, 2008).

Initial consideration regarding identification of chemical messengers began in 1902 when Ernest Starling and William Bayliss introduced the existence of an internal communication system with their discovery of the first hormone, secretin (Zarate and Saucedo, 2005). Subsequently, scientists have identified numerous other chemical messengers within the body such as epinephrine

* Corresponding author. Tel.: +1 715 294 2144; fax: +1 715 755 2267.
E-mail address: david.marc@neurorelief.com (D.T. Marc).

and norepinephrine (Henderson, 2005). The measurement of chemical messengers was rapidly adopted as a means to assess functions of organs or tissues and became the basis for diagnostic or functional indicators in clinical practice. Despite a historical absence of relevant biomarkers in the realm of clinical psychiatry, this format has expanded (Cook, 2008; Wong et al., 2002) and neurotransmitters now serve as a primary target for the development of predictive or correlative biomarkers of nervous system function (Wong et al., 2002). Therefore, the following review provides a summary of the scientific evidence on the validity, feasibility, and clinical utility of urinary neurotransmitter testing.

1.1. History of urinary neurotransmitter measurement

Neurotransmitters are present throughout the body and represented by research demonstrating measurements in various biological fluids, including serum, plasma, platelets, cerebral spinal fluid (CSF), saliva, and urine (Roy et al., 1988; Okumura et al., 1997). Urine, due to its non-invasive method of collection, and being the primary method of neurotransmitter elimination, has been the preferred bodily fluid for neurotransmitter measurements (Lepschy et al., 2008). For nearly 60 years, studies have utilized urinary measurements of neurotransmitters and neurotransmitter metabolites. Since the 1950s, increased urinary excretion of dopamine (DA), norepinephrine (NE), and epinephrine (E), the three naturally occurring catecholamines, has been used to diagnose pheochromocytoma (Duncan et al., 1988; Engel and von Euler, 1950; Moyer et al., 1979; Rosano et al., 1991). Pheochromocytoma is a rare tumor of the adrenal gland medulla resulting in the overproduction of catecholamines with accompanying hypertension (Westphal, 2005). In accordance with this, studies conducted at the Mayo Clinic in Rochester, MN, suggested that 24-h urinary metanephrine, an E metabolite, and catecholamine measurements are the tests of choice for diagnosing pheochromocytoma in a clinical setting to avoid excessive false-positive results in a particular low-risk population (Kudva et al., 2003).

Since the inception of urinary neurotransmitter testing, methods for measuring catecholamines have been improved in both the sensitivity and specificity. Early catecholamine research was hampered by limitation with colorimetric and bioassays that lacked adequate sensitivity and specificity (Kagedal and Goldstein, 1988). Technology then progressed with the development and utilization of fluorometric methods. More recently, high-performance liquid chromatography (HPLC) methodology has greatly enhanced the specificity and sensitivity of these measurements and has allowed for larger-scale clinical applications (Kagedal and Goldstein, 1988; Westermann et al., 2002; Panholzer et al., 1999). Enzyme-linked-immunosorbent assay (ELISA) and radioimmunoassay (RIA) technologies offer the greatest methodological alternatives, allowing for higher-throughput, increased sensitivity and specificity, and reduced cost (Westermann et al., 2002; Huisman et al., 2010; Francis et al., 2010). Therefore, advancement in the methodology has enhanced not only the research potential but also the feasibility of introducing neurotransmitter measurement into a clinical setting due to high-throughput and low costs.

1.2. Neurotransmitter transport and the blood–brain barrier

Neurotransmitters serve specific biological functions throughout the body; however, the important role of neurotransmitters in regulating neurological function in the brain has led to significant research focusing on the movement of neurotransmitters to and from the brain. Passage of molecules in and out of the central nervous system (CNS) is highly regulated by the blood–brain barrier (BBB) (Ballabh et al., 2004; Rubin and Staddon, 1999). Three cellular elements of the brain microvasculature comprise the BBB: endothe-

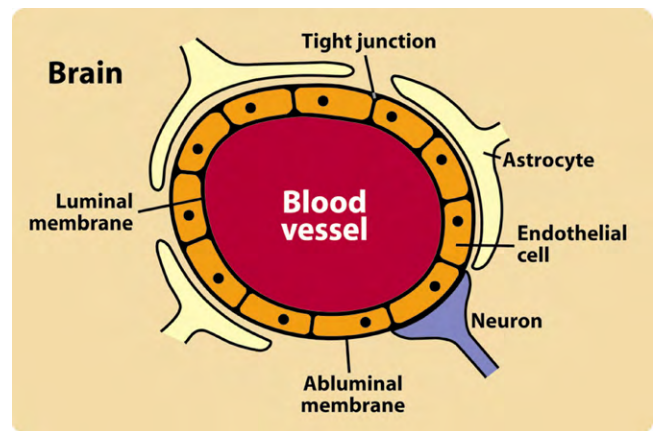


Fig. 1. The blood–brain barrier is composed of endothelial cells, astrocytes, and pericytes. The endothelial cells are connected by tight junctions and polarized into luminal (blood-facing) and abluminal (brain-facing) plasma membrane domains. The endothelial cell layer possesses specific transporters that regulate passage of neurotransmitters in and out of the CNS.

lial cells, astrocyte end-feet, and pericytes (PCs) (Ballabh et al., 2004). Located within the capillaries that deliver blood to the brain, the BBB is a single layer of specialized endothelial cells referred to as brain capillary endothelial cells (BCECs) (Rubin and Staddon, 1999). BCECs are connected by highly resistant tight junctions (Rubin and Staddon, 1999), and are polarized into luminal (blood-facing) and abluminal (brain-facing) plasma membrane domains (Hawkins et al., 2006; see Fig. 1). The selective permeability of BCECs serves a protective role by limiting the passage of molecules into the CNS thus preventing many disruptive or harmful molecules from entering the brain (Pardridge, 1999). Some molecules, however, are transported into the CNS, such as amino acids obtained from the diet, which are then synthesized into neurotransmitters in brain neurons (Fernstrom and Fernstrom, 2007; Hawkins et al., 2006; Wurtman, 1987, 1988). Other molecules have the ability to be transported out of the CNS into the periphery. It is a commonly held misconception that molecules, such as neurotransmitters, cannot be transported from the CNS to the periphery (Ohtsuki, 2004). To the contrary, a large body of research has demonstrated that BCECs possess specific transporters that regulate the passage of neurotransmitters out of the CNS (Hawkins et al., 2006; Ohtsuki, 2004; Rubin and Staddon, 1999; Tamai and Tsuji, 2000; see Table 1).

The BBB transporters that shuttle neurotransmitters and their metabolites out of the CNS are depicted in Fig. 2. Studies have documented the presence of a number of major neurotransmitter transporters at the BBB, such as the serotonin transporter (SERT) (Nakatani et al., 2008), norepinephrine transporter (NET) (Wakayama et al., 2002), GABA transporter (GAT) (Takanaga et al., 2001), and excitatory amino acid transporter (EAAT) (Hawkins et al., 2006). Other studies have demonstrated the ability of neurotransmitters to cross cerebral endothelial membranes, further supporting intact CNS neurotransmitter efflux (Husztli et al., 1995; Szabo et al., 2001; see Table 1). As shown in Table 1, transporters located either on the abluminal and/or luminal membranes allow the facilitated transport of specific neurotransmitters from the brain to the blood. In particular, transporters identified on the abluminal membrane transport GABA, dopamine, norepinephrine, serotonin, glutamate, aspartic acid, and histamine from the brain into the BCECs. Likewise, transporters identified on the luminal membranes allow the transport of GABA, glutamate, aspartic acid, and histamine out of the BCECs into the blood with bidirectional transport of glutamate, aspartic acid, and histamine. Serotonin transported via the serotonin transporter (SERT) is taken from the blood into the BCECs. Lastly, agmatine and PEA can freely cross

Table 1

Various different transporters found on brain capillary epithelial cells (BCECs) that line the blood–brain barrier (BBB) regulate the transport of neurotransmitters out of the brain.

Neurotransmitter	Transport mechanism	References
Gamma-aminobutyric acid (GABA),	Transport out of the brain occurs on the abluminal membrane via the GAT2/BGT-1 transport system. GABA transport across the luminal membrane is thought to occur, however, the transporter has not yet been identified	Takanaga et al. (2001), Kakee et al. (2001)
Dopamine	Has been found to cross the abluminal membrane via the norepinephrine transporter (NET)	Ohtsuki (2004), Wakayama et al. (2002)
Norepinephrine	Has been found to cross the abluminal membrane via the NET	Ohtsuki (2004), Wakayama et al. (2002)
Epinephrine	Uptake in human endothelial cells has been demonstrated in vitro	Hardebo and Owman (1980)
Serotonin	Has been found to cross both the luminal and abluminal membranes via the serotonin transporter (SERT). Transport is in the direction of brain to BCEC on the abluminal membrane whereas luminal transport is thought to be bidirectional	Wakayama et al. (2002), Nakatani et al. (2008)
Glutamate	Actively transported on the abluminal membrane into the endothelial cell via excitatory amino acid transporters (EAAT, EAAT1, EAAT2, and EAAT3). These transporters not only actively transport glutamate out of the brain, but restrict entry of glutamate into the brain. The facilitative glutamate transporter, XG-, is located on the luminal membrane, allowing for bidirectional transport of glutamate	Arriza et al. (1994), Hawkins et al. (2006)
Aspartic acid	Transported out of the brain across the abluminal membrane via the EAAT and ASCT2. Luminal transport is facilitated by XG-	Hosoya et al. (1999), Hawkins et al. (2006)
Phenylethylamine (PEA)	Lipid-soluble neurotransmitter that freely crosses the blood–brain barrier	Szabo et al. (2001)
Histamine	Has been shown to cross both abluminal and luminal cerebral endothelial membranes, with primary action being luminal release	Husztai et al. (1995)

The BCECs are polarized into luminal and abluminal membranes and the transporters can be found on either of these membranes.

the BBB due to their lipophilicity (see Table 1; see Fig. 2). Based on this information, evidence supports a blood–brain transport mechanism that is substrate specific and can be either unidirectional or bidirectional.

1.3. Neurotransmitter transport and the kidneys

This review presents information that elucidates the role of the kidneys' neurotransmitter transport mechanisms, nephron filtration, active transport, intrakidney synthesis, and nerve innervation as contributors to the total urinary neurotransmitter pool. Circulating neurotransmitters are filtered from the blood by nephrons, the functional units in the kidneys, and subsequently excreted in the urine (Moleman et al., 1992). Studies have identified the presence of neurotransmitter transport molecules on nephrons that move neurotransmitters from the extracellular space to abolish their biological actions and actively excrete them in the urine (Amara and Kuhar, 1993; Grundemann et al., 1998, 1997; Chen et al., 2004; Hayer-Zillgen et al., 2002; Kopp et al., 1983). Among these, the organic cation transporters (OCTs) are important facilitators of electrogenic uptake of small cations such as drugs, xenobiotics, and endogenous compounds (choline, DA, NE, E, serotonin, histamine, and tyramine) from the circulation, and are present in proximal convoluted tubules of the nephrons (Grundemann et al., 1998; Karbach et al., 2000). A notable property of OCTs is the concentration dependent reversibility of the transport direction thereby facilitating the bidirectional transport of organic cations (Busch et al., 1998; Gorboulev et al., 1997; Kekuda et al., 1998; Koepsell et al., 1999).

Three OCT subtypes exist, including OCT3 (Kekuda et al., 1998), OCT2 (Grundemann et al., 1998), and OCT1 (Busch et al., 1996; Kekuda et al., 1998). The OCT3 subtype is primarily expressed in the kidney and brain tissue (Wu et al., 2000). Specifically, OCT3 is localized in the hippocampus, cerebellum, and cerebral cortex and mediates the uptake of DA in mammalian cells (Wu et al., 1998). The OCT2 subtype has been characterized in human embryonic kidney cells (Grundemann et al., 1997; Kekuda et al., 1998) and is involved in transmembrane transport of choline, DA, NE, E, serotonin, histamine, and tyramine (Grundemann et al., 1998; Kekuda et al., 1998). Immunohistochemical experiments suggest

that human OCT2 is localized in the kidneys at the basolateral membrane of the proximal convoluted tubules (Karbach et al., 2000). Additionally, OCT2 is found in specific brain regions that are rich in DA including the nucleus accumbens, striatum, and substantia nigra (Grundemann et al., 1997). Similar to OCT2, OCT1 is expressed in the kidneys as well as the liver where it can transport tyramine, epinephrine, dopamine, serotonin, and norepinephrine (Breidert et al., 1998). OCT1 provides bidirectional, pH-dependent, transport to multiple organic cations to facilitate the elimination of such biochemicals (Yabuuchi et al., 1999). Recently, a novel transporter was identified, termed plasma membrane monoamine transporter (PMAT or ENT4). PMAT is expressed in the brain, skeletal muscle, liver, kidney, and heart (Engel and Wang, 2005). PMAT is a Na⁺-independent and membrane potential-sensitive transporter, which shares functional similarities with OCTs and also shuttles the monoamine neurotransmitters from the blood to the kidneys (Grundemann et al., 1997; Engel and Wang, 2005). Altogether, OCT and PMAT mediated transport of specific neurotransmitters from circulation into the kidneys provides an important mechanism to regulate levels of neurotransmitters in the blood and excretion into the urine.

Neurotransmitters are taken from systemic circulation, transported into the kidneys, and then excreted into the urine by active transport via OCTs as well as by glomerular filtration. These two mechanisms of neurotransmitter transport in the kidneys have been well established: (1) monoamine neurotransmitters are excreted by ultrafiltration from arterial blood into the glomeruli, secreted in the proximal tubules, subsequently distributed through the collecting duct to the urinary bladder and excreted in the urine (Graefe et al., 1997), (2) in the luminal and basolateral membranes of the renal proximal tubules, OCT2 is responsible for the reabsorption and secretion of endogenous compounds, including monoamine neurotransmitters (Koepsell et al., 1998) (Fig. 3.). This is achieved by electrogenic uptake of monoamine neurotransmitters at the basolateral membrane via OCTs and cation release at the luminal membrane of proximal tubular epithelial cells mediated by an electroneutral proton cation antiporter (Koepsell et al., 1998). Research has demonstrated that choline and other cations may be reabsorbed from the proximal tubule through OCTs and returned to circulation when concentrations are low in the plasma

(Acara and Rennick, 1973). Hence, cations localized at the proximal tubule can be transported to the plasma to compensate for low circulating cation levels thereby providing a regulatory mechanism to maintain sufficient cation levels in circulation.

Disprocynium24, a potent inhibitor of extraneuronal monoamine transport, was shown to block OCTs thereby reducing the overall clearance of catecholamines from plasma (Wu et al., 1998). Urine collections obtained from anesthetized animals treated with Disprocynium24 exhibited marked decreases in urinary DA, NE, and E (Acara and Rennick, 1973; Graefe et al., 1997) with increased plasma concentrations of NE, E, normetanephrine and metanephrine (Eisenhofer et al., 1996). This observation demonstrated that Disprocynium24 decreased catecholamine clearance from plasma (Graefe et al., 1997). Based on this research, it is apparent that the level of catecholamines in urine is dependent

upon plasma concentration and uptake from OCTs (Eisenhofer et al., 1996; Graefe et al., 1997). Inhibition of OCT with Disprocynium24 can lead to decreased urinary catecholamine excretion with subsequent increases in plasma catecholamine levels (Graefe et al., 1997). Therefore, urinary neurotransmitter excretion mediated by OCT, is dependent on circulating neurotransmitter concentrations (Chekhonin et al., 2000; Davis et al., 1978; Lynn-Bullock et al., 2004; Seegal et al., 1986; Westermann et al., 2002).

Although neurotransmitters that are taken up via OCTs contribute largely to the overall urinary neurotransmitter pool, other mechanisms also offer contributions. Studies have suggested that human kidneys contain enzymes necessary to synthesize and metabolize monoamine neurotransmitters. This process, similar to neurotransmitter synthesis in the brain, appears to be dependant on precursor availability and studies have reported parallel changes

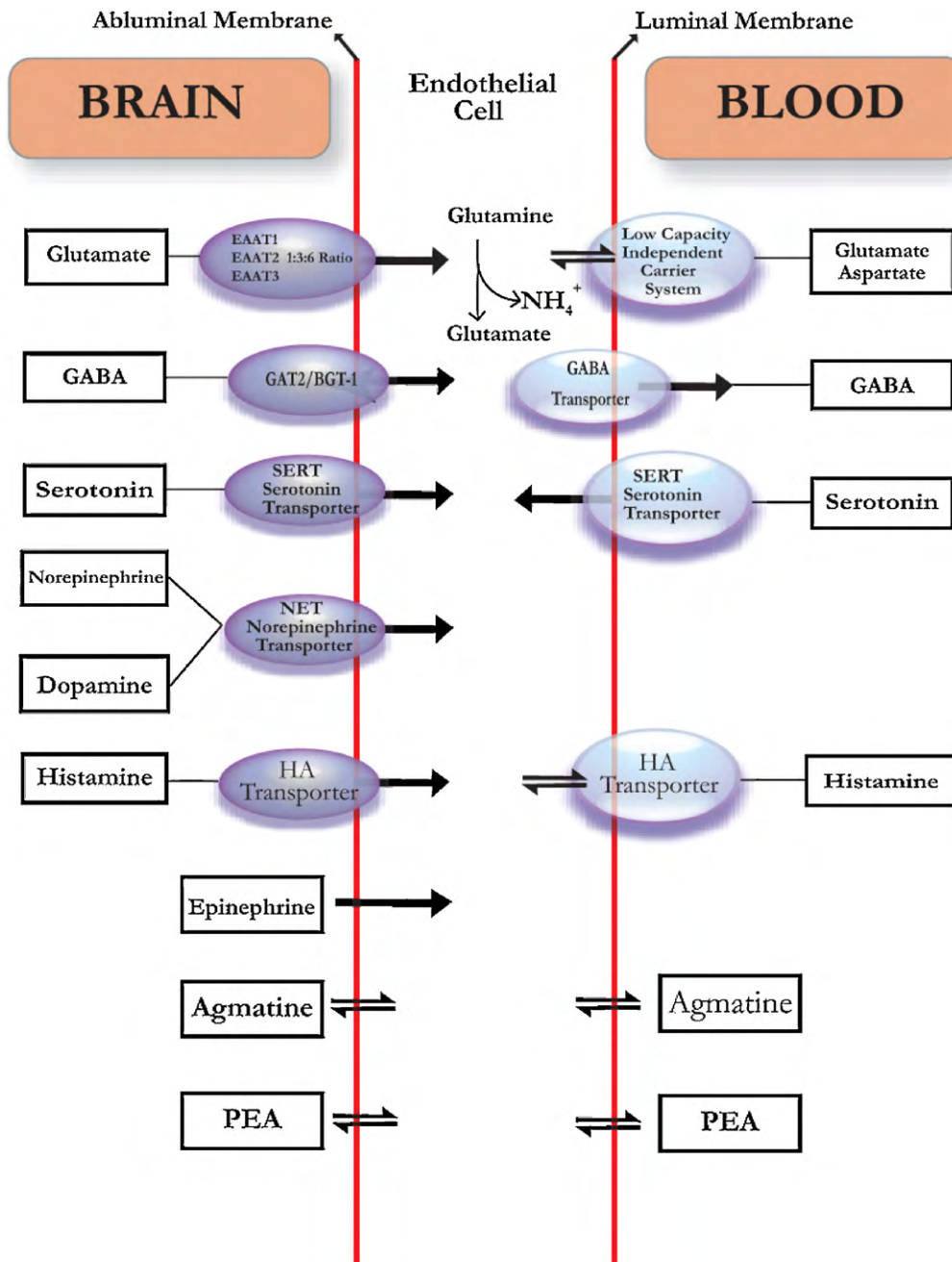


Fig. 2. Neurotransmitter transport across the blood–brain barrier is mediated by specific transporters. The transporters are specific for various neurotransmitters allowing access from the brain to the blood and vice versa.

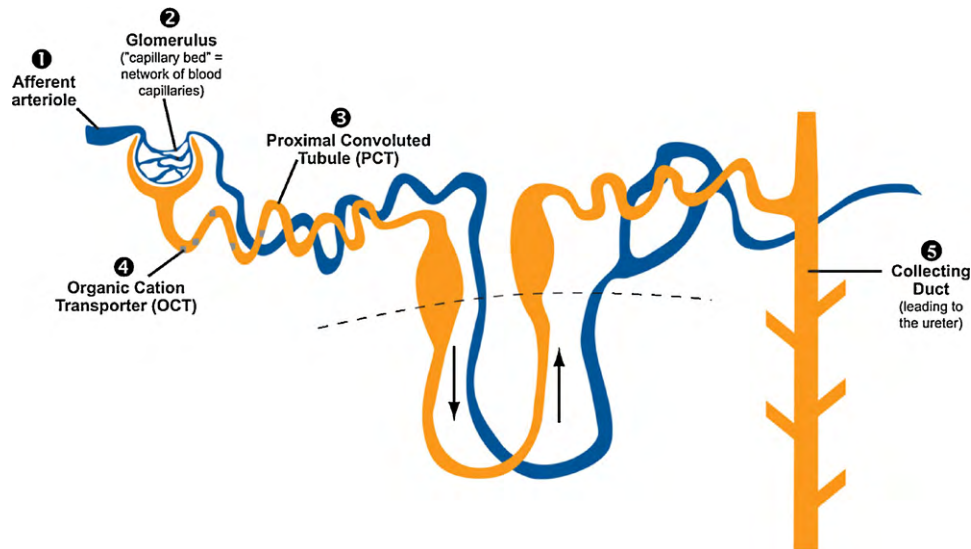


Fig. 3. The nephron is the functional unit of the kidney and can transport neurotransmitters in specific ways. There are two methods for neurotransmitter transport to the urine. 1) Drugs, xenobiotics, and organic endogenous compounds, including neurotransmitters, are filtered from systemic circulation into the kidneys via afferent arteriole blood supply to a nephron (1). Afferent arterioles blood passes through the kidney glomerulus where neurotransmitters are filtered into the Bowman's capsule (2). The neurotransmitters are secreted into the proximal convoluted tubules (3), pass through the loop of henle to the distal convoluted tubule, excreted to the collecting duct (5) and released into the urinary bladder until urination. 2) Specific organic cation transporters (OCTs) (4) are present in the proximal convoluted tubule that can also facilitate movement of neurotransmitters from circulation by transporting neurotransmitters from peritubular capillary blood into proximal convoluted tubules. Interestingly, OCTs have the ability to reverse the transport direction of substrates so that neurotransmitters can be transported from proximal convoluted tubules and recycled back into circulation.

during serotonin and dopamine prodrug treatment (Wa et al., 1995; Dantonello et al., 1998). The kidneys also receive neural input from sympathetic nerves, which can release norepinephrine and dopamine into the kidneys thereby adding an additional process that potentially influences urinary neurotransmitter concentrations (Wa et al., 1995; Noshiro et al., 1991). The degree to which renal neurotransmitter synthesis can influence urinary neurotransmitter levels must be considered as a potential factor that can contribute to the total urinary pool of neurotransmitters. A study that examined renal catecholamine excretion demonstrated how kidney nerve stimulation led to a slight increase in urinary catecholamine output (Kopp et al., 1983). However, based on the data collected, the researchers concluded that urinary catecholamine excretion was a poor indicator of renal nerve catecholamine release (Kopp et al., 1983).

Conclusive information that has evaluated the contribution of central and peripheral output to the urinary excretion of neurotransmitters is scant. One attempt by Graefe et al. (1997) quantified the level of catecholamine excretion in urine derived from plasma by measuring excretion of radiolabeled catecholamine levels in urine divided by the level of radiolabeled catecholamines in plasma. They concluded that 100% of urinary E, 68% of NE, and 2.3% of DA stemmed from circulation (Graefe et al., 1997). The remainder of the NE is from renal nerve release while the remainder of DA is from multiple sources including: renal DA synthesis, release of DA from renal nerves, and DA formed by the de-conjugation of circulating DA conjugates (Graefe et al., 1997). While unconfirmed, these findings demonstrate how the renal contribution to overall neurotransmitter excretion can vary for each neurotransmitter and highlights the value of urine as a means to evaluate system-wide disturbances in neurotransmitter function.

1.4. Urinary neurotransmitters and CNS associations

Although it is not fully understood if there is a direct association between neurotransmitter levels in the CNS and neurotransmitter levels excreted in the urine, some studies have suggested a link (Matsuda et al., 1991; Chekhonin et al., 2000; Lynn-Bullock

et al., 2004). Early research by Maas and Landis (1965) described a potential route of NE transport from the brain to the urine. They investigated the kinetics of NE metabolism in the brain of dogs by injecting H^3 NE into the cisterna magna, which is an opening in the subarachnoid space between the arachnoid and pia mater layers of the meninges surrounding the brain. After injecting H^3 NE into the cisterna magna, the researchers observed a significant amount of H^3 NE in blood with rapid clearance from the blood into the urine (Maas and Landis, 1965). Based on these observations, the authors proposed that urinary H^3 NE and H^3 NE metabolites originated at least in part from the CNS (Maas and Landis, 1965).

Further animals studies conducted in rats examined the effects of oral ingestion of the serotonin substrate, 5-hydroxytryptophan (5-HTP), on specific brain regions (Lynn-Bullock et al., 2004). Serotonin is a monoamine neurotransmitter and is implicated in many physiological and behavioral functions including: affect, aggression, appetite, cognition, emesis, endocrine function, gastrointestinal function, motor function, neurotrophism, perception, sensory function, sex, sleep, and vascular function (Bloom and Kupfer, 1995; Lynn-Bullock et al., 2004). Serotonin levels were measured using brain tissue immunoreactivity and urinalysis and observed maximum serotonin immunoreactivity in the serotonergic dorsal raphe nucleus within 2 h of 5-HTP administration. Urinary analysis of serotonin, 5-HTP, and 5-hydroxyindolacetic acid (5-HIAA), a serotonin metabolite, had parallel changes in immunoreactivity to the dorsal raphe nucleus, demonstrating a positive correlation between CNS serotonergic activity and urinary serotonin levels (Lynn-Bullock et al., 2004) and possible parallels in the mechanisms regulating CNS and urinary neurotransmitter levels.

Another study in rats investigated the effect of 6-hydroxydopamine (6-OHDA) induced hemiparkinsonism on the metabolism of catecholamines, as well as the relationship between cerebral catecholamine content and urinary catecholamine excretion (Chekhonin et al., 2000). Chekhonin et al. (2000) induced hemiparkinsonism, as identified when Parkinson symptoms present unilaterally, following the injection of the neurotoxic compound 6-OHDA into the rat striatum. A positive

correlation between urinary and striatal DA concentrations was reported. The researchers concluded that the measurement of urinary catecholamines and their metabolites might be a biomarker for evaluating the status of the dopaminergic nigrostriatal system of the brain in experimental parkinsonism (Chekhonin et al., 2000).

A limitation consistent throughout these studies is the lack of human data. In the absence of human studies comparing urinary neurotransmitter output to central nervous system activity, researchers have attempted to correlate urine and cerebral spinal fluid (CSF) neurotransmitter levels in humans (Castellanos et al., 1994; Swann et al., 1987; Koslow et al., 1986). While vast amounts of research has examined correlations between CSF and various conditions, a review of the literature did not reveal any studies that suggested CSF measurements were viable biomarkers for any psychological condition. Nor is there conclusive evidence that has suggested CSF may be a superior clinical correlate to urine (Roy and Pollack, 1994). Roy and Pollack (1994) found that urinary measures of DA metabolite had a stronger association with suicide attempts in depression than CSF. Additionally, a primary consideration regarding the utilization of CSF in a clinical setting is the inherent risk associated with the invasive collection procedure. The mental, physical, and emotional stressors that accompany CSF collection can activate neurological stress pathways and thereby alter neurotransmitter responses (Nigrovic et al., 2007). Therefore, proper collection techniques are crucial for CSF collection to prevent a stress response from potentially skewing the results obtained (Manyam and Hare, 1983; Wood, 1980).

Overall, the above research suggests a relationship between urinary neurotransmitter measurements and CNS levels. At present time, the exact amount of CNS neurotransmitters that contribute to the overall urinary pool is unknown. Due to the presence of the blood–CNS barrier limiting the transport of neurotransmitters from the periphery to the CNS, many believe that peripheral markers, such as plasma or urinary neurotransmitters, are poor indicators of CNS function (Ohtsuki, 2004). Others have suggested that the association between neurotransmitters in the periphery and neurotransmitters in the CNS can be understood through the neuronal crosstalk between the brain and the rest of the body (Lechin et al., 1996). Lechin et al. (1996) provided an explanation regarding the relationship between the CNS and PNS neurotransmitters to describe how the measurement of peripheral activity can provide clinical insight into CNS profiles and aid in treatment selection. They propose that many links exist that connect the brain and body including neurotransmitters released by peripheral neurons and glandular cells (adrenal, enterochromaffin cells, mast cells), which together are released into the bloodstream. They go on to say that although neurotransmitters cannot readily enter the brain, basic and clinical research has established the relationship between CNS and PNS neurotransmitter activities. Hence, Lechin et al. (1996) suggest that it is possible to obtain some information regarding CNS function through the measurement of circulating neurotransmitters.

Lechin and others have clearly illustrated the relationship and crosstalk between the CNS and PNS (Gallowitsch-Puerta and Pavlov, 2007; Lechin and van der Dijs, 2006; Mayer et al., 2006). Future research, however, should expand our understanding of the specific CNS circuits that contribute to particular peripheral neurotransmitter alterations.

Although a direct association between neurotransmitters in the CNS and those excreted in the urine is not yet defined, the value of urinary excreted neurotransmitters as biomarkers of the nervous system may be best evaluated by examining correlations to various clinical conditions. Interestingly, most research that has examined the clinical utility of urinary neurotransmitter testing has focused on only a few disorders: depression, attention deficit hyperactivity disorder (ADHD), and inflammation have been the primary focus

of research to attempt to delineate the clinical utility of urinary neurotransmitter assessment.

1.5. Urinary neurotransmitters and depressive symptoms

In the years since von Euler published data on the excretion of urinary neurotransmitters and their metabolites (von Euler and Luft, 1951; von Euler and Hellner, 1951), numerous studies have tested urinary neurotransmitter levels in various psychological disorders (Manyam and Hare, 1983; von Euler et al., 1955; von Euler and Luft, 1951; Wood, 1980). Specifically, studies suggested urinary neurotransmitter assessments might be a viable means to describe a disease state and to monitor therapeutic interventions (Baker et al., 1991; Delahanty et al., 2005; Hughes et al., 2004; Mooney et al., 2008; Otte et al., 2005; Kotzailias et al., 2004).

In line with these studies, research has described the utility of urinary neurotransmitter analysis in bipolar depression (Koslow et al., 1983) and subtypes of unipolar depression (Schildkraut et al., 1978). Specifically, patients that met the criteria for a major depressive episode had overall higher levels of urinary NE than controls (Roy et al., 1986a,b). Similarly, Koslow et al. (1983) found that compared with controls, depressed patients had significantly higher levels of urinary NE along with E and their metabolites. The researchers concluded that total body catecholamine turnover (measurement of urinary catecholamines and their metabolites) might provide more useful information than metabolite measures alone (Koslow et al., 1983). Additionally, higher urinary NE levels were found to distinguish unipolar from bipolar depressed patients (Koslow et al., 1983). Further studies have since confirmed the positive relationship with urinary NE and depression (Hughes et al., 2004; Roy et al., 1986a,b; Grossman and Potter, 1999), while other studies have revealed an overall elevation in urinary catecholamines in unipolar depressed patients compared to bipolar patients (Joyce et al., 1995; Maas et al., 1987; Schatzberg, 1998; Schildkraut et al., 1978). More recently, Hughes et al. (2004) observed that higher levels of depressive symptoms, as assessed by the Beck Depression Inventory, were associated with increased NE excretion in the urine. Symptoms of anxiety, as assessed by the state anxiety portion of the Spielberger State-Trait Anxiety Inventory, were also associated with increased NE excretion in the urine (Hughes et al., 2004). The investigators concluded that in depression and anxiety, increased sympathetic nervous system (SNS) activity contributed to an increased rate of mortality in these patients (Hughes et al., 2004; Otte et al., 2005).

While urinary neurotransmitter assessment has identified biochemical differences, it is yet to be accepted as diagnostic for depression or any particular disease or condition. In the future, it is possible that the accumulative affects of information coming together, such as current diagnostic protocols along with neurotransmitter assessment, will allow urinary neurotransmitters to serve a diagnostic role. As of now, the use of urinary testing of neurotransmitters as a biomarker may be best applied to guide therapeutic decisions and assist practitioners in clinical settings to effectively predict treatment responses (Holsboer, 2008). Many diseases are treated with nutritional, pharmaceutical, and electrical interventions that affect neurotransmitters. Unfortunately, due to the significant comorbidity of neurotransmitter related diseases and the spectral nature of the underlying neurotransmitter imbalances that trigger them, treatment success is often difficult to achieve or identify (Benazzi, 2006; Nemeroff, 2007). Screening tools are currently being sought after to guide the selection of specific interventions and improve patient response rates (Le-Niculescu et al., 2008; Schwarz and Bahn, 2008). Therefore, urinary neurotransmitter measurements may be a valuable tool in the assessment and subsequent management of a vast number of clinical conditions.

As seen in patients with depression, studies have demonstrated that low urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), a NE metabolite, predicts a positive response to NE-selective drugs such as imipramine, nortriptyline, desipramine, or maprotiline (Mooney et al., 1991, 2008; Schildkraut et al., 1992). Subjects with high urinary MHPG predicted a positive response to the benzodiazepine alprazolam (Mooney et al., 1985, 1988) and a poor response to imipramine, nortriptyline, desipramine, and maprotiline (Mooney et al., 1991, 2008; Schildkraut et al., 1992). These studies therefore, illustrate the significance of urinary neurotransmitter measurements in guidance of treatment selection and the prediction of efficacy.

In addition to the use of urinary testing as a guide for therapeutic decisions, the methodology may further apply to monitoring pharmaceutical interventions. Longitudinal analysis of urinary catecholamines and metabolites has revealed that desipramine, a NE reuptake inhibitor, increased urinary NE levels and decreased MHPG levels (Schildkraut et al., 1992). It was later described that desipramine increased intact NE excretion in the urine and reduced the excretion rate of metabolites (Schatzberg, 1998). As such, limited but promising data has demonstrated that urinary neurotransmitter analysis can reflect changes caused by psychotropic medications and monitor treatment effectiveness in depressed patients.

1.6. Urinary neurotransmitters and ADHD

Urinary neurotransmitter analysis has been utilized to assess neural biochemistry in relation to symptoms of attention-deficit hyperactivity disorder (ADHD) (Hanna et al., 1996; Kusaga et al., 2002). Patients with ADHD were shown to have alterations in urinary excretion of β -phenylethylamine (PEA) and the catecholamines (Hanna et al., 1996; Kusaga et al., 2002). Importantly, PEA is a monoamine neurotransmitter that has amphetamine-like functions that can alter mood and attention (Berry, 2004). Decreased PEA levels have been associated with symptoms of inattentiveness (Berry, 2004). Urinary PEA levels were significantly lower in ADHD patients compared with normal controls, indicating that disturbances in PEA contribute to decreased attention and focus (Baker et al., 1991; Kusaga et al., 2002). Furthermore, lower levels of urinary E and 3,4-dihydroxyphenylglycol (DOPEG), a NE metabolite, were found in a population of adolescent males diagnosed with ADHD, suggesting abnormal metabolism of NE and E in ADHD (Hanna et al., 1996). Higher urinary E excretion was also observed in subjects with comorbid ADHD and anxiety (Pliszka et al., 1994). Taken together, these findings suggest that sympathoadrenomedullary function may be altered in subjects with ADHD (Anderson et al., 2000).

A common mode of intervention for ADHD is the use of stimulant medications (Cormier, 2008). Research has reported changes in urinary neurotransmitter levels in conjunction with stimulant use in ADHD subjects (Hermens et al., 2006). After treatment with methylphenidate, a stimulant that inhibits the reuptake of NE and DA, urinary PEA levels significantly increased in responders to the medication, whereas non-responders did not demonstrate a significant change (Kusaga et al., 2002). Additionally, methylphenidate treatment has shown to increase urinary E levels (Elia et al., 1990). Interestingly, methylphenidate's effects on neurotransmitter excretion were shown to be different from dextroamphetamine, another CNS stimulant. Urinary NE and normetanephrine (NMN), a NE metabolite, were significantly elevated following methylphenidate administration, whereas following dextroamphetamine treatment, MHPG excretion was reduced with no changes in NE and NMN (Zametkin et al., 1985; Zametkin and Hamburger, 1988). These findings illustrated the utilization of urinary neurotransmitter measurements to deter-

mine the underlying biochemical imbalances that exist in subjects with ADHD to ensure appropriate treatment selection and monitor treatment effectiveness.

1.7. Urinary neurotransmitters and inflammation

Historically, clinicians have utilized urinary histamine (HIST) measures as a means to monitor recurrent anaphylactic reactions (Hershko et al., 2001; Tang, 2003; Yamatodani, 1990). Chemicals such as HIST, prostaglandins, and leukotrienes are released by degranulation from basophil and mast cells during an anaphylactic reaction (Schwartz, 2004) and can be detected in urine and plasma (Hogan and Schwartz, 1997). Circulating HIST is excreted by the kidneys intact, allowing for the utilization of urinary measures to monitor fluctuations in plasma HIST with the advantage of greater stability and accessibility (Myers et al., 1981). In addition, research has shown that urinary HIST levels can be useful in the assessment of inflammatory conditions other than anaphylaxis (Asano et al., 1995; Hershko et al., 2001; Nishiwaki et al., 2000; Skoner et al., 2001). Asthma, a condition in which airways become inflamed and constricted causing difficulty in breathing, has been evaluated using urinary HIST (Asano et al., 1995; Nishiwaki et al., 2000). Some studies showed no significant changes in diurnal variation of urinary HIST with moderate-to-severe asthmatic patients compared to controls (Asano et al., 1995). Other studies have revealed increased concentrations of urinary HIST and 1-methylhistamine, a histamine metabolite, after asthma attacks (Nishiwaki et al., 2000). The difference in findings may be due to the proximity of urine collection to an asthma attack.

Research has also examined urinary HIST in subjects with interstitial cystitis, a urinary bladder disease characterized by increased urination frequency. Urinary HIST and urinary interleukin-6 levels were significantly greater in subjects with interstitial cystitis than controls (Lamale et al., 2006). Nanocrystalline silver (1%) may be a useful anti-inflammatory intervention for interstitial cystitis as evidenced by decreased urinary HIST, tumor necrosis factor- α (TNF- α) and mast cell activation after administration (Boucher et al., 2008). Furthermore, in subjects with systemic mastocytosis, treatment with interferon α -2b therapy caused a decrease in urinary and serum HIST (Hubner et al., 1997; Takasaki et al., 1998). This body of evidence suggested urinary HIST measurements can provide a non-invasive method of assessing anaphylactic reactions along with other inflammatory conditions as well as monitoring immunomodulatory treatments (Murdoch et al., 1993).

The actions of other neurotransmitters have also been examined for their role in inflammatory processes. Elenkov and Chrousos (2002) describe a mechanism in which NE and E can modulate levels of cytokines by acting on adrenergic receptors located on immune cells. In a review by Elenkov et al. (2009), the authors illustrate that NE and E have immunomodulatory actions by fine tuning immune responses. These catecholamines may exert pro- and anti-inflammatory effect depending on the nature of the antigens that initiate the immune response and/or the presence of specific receptor subtypes located on immune cells (for review see Elenkov et al., 2009). At the present time, studies have not examined the relationship between urinary NE and E and inflammation. Based on the current information that has established a relationship between the catecholamines and immunomodulation, future research should be conducted to determine if urinary catecholamine alterations exist with inflammatory processes.

1.8. Limitations of urinary neurotransmitter assessment

A primary limitation of urinary neurotransmitter assessment is that neurotransmitters, in any medium, are not recognized as diag-

nostic for any particular disease or condition, with the exception of pheochromocytoma (Duncan et al., 1988; Westphal, 2005). Unless further research can elucidate diagnostic capabilities, urinary neurotransmitter technology will remain a functional assessment. A second limitation concerns the insufficient data regarding the origin of urinary neurotransmitters. Neurotransmitters are synthesized in the CNS and most organs in the body, thus, multiple systems contribute to the total urinary pool of neurotransmitters (Eisenhofer et al., 1996, 2004). This presents a challenge in interpreting urinary transmitter data. Further studies are needed to elucidate the relationship between the CNS and PNS and how CNS neurotransmitter activity influences the total urinary neurotransmitter pool. Similarly, studies are needed to evaluate how factors (i.e. medications and supplements) that alter neurotransmitter levels in the CNS affect neurotransmitters in the urine and vice versa.

In terms of clinical efficacy, neurotransmitters can act as biomarkers for treatment selection and treatment outcome for psychiatric and inflammatory disorders (Wong et al., 2002). Additional research is needed that focuses on the use of urinary neurotransmitter analysis in predicting treatment efficacy using psychotropic and immune-modulating medications. In addition, with the development of neurocircuitry models (Lechin and van der Duijn, 2006), urinary neurotransmitter analysis may provide further insight into specific CNS pathways that contribute to changes in circulating neurotransmitter levels. Therefore, urinary neurotransmitter assessments may prove to be a useful clinical tool to better understand peripheral abnormalities that result from neurological changes in specific brain pathways.

2. Conclusion

Because urinary assessments are non-invasive, with the added advantage of enhanced stability compared to CSF or blood, the concept of neurotransmitter measurements as an objective means to assess nervous system function serves as a viable option for the clinician addressing neuropsychiatric health concerns. The current body of literature provides evidence that neurotransmitters excreted in the urine may have a place in clinical practice as biomarkers of nervous system function. Urinary neurotransmitter measurements were initially utilized to diagnose pheochromocytoma (Duncan et al., 1988), but with progressive research, neurotransmitter testing has shown promise as a method to evaluate patients with psychiatric and inflammatory disorders (Delahanty et al., 2005; Otte et al., 2005; Anderson et al., 2000). In support of urinary neurotransmitter assessment, studies have demonstrated that intact neurotransmitter are transported from the CNS to the periphery, via specific BBB transporters, followed by renal filtration of neurotransmitters with subsequent excretion in the urine. Additionally, animal studies have suggested a relationship between neurotransmitters excreted in the urine and neurotransmitters in the CNS (Lynn-Bullock et al., 2004). Currently, there is limited data that examines the association between CNS and urinary neurotransmitter in humans. As such, this scientific gap poses a significant limitation in the feasibility of urinary neurotransmitter testing in predicting precise CNS function and needs to be addressed with future investigations. Lastly, this review demonstrated that many studies have revealed associations between urinary neurotransmitters and various clinical conditions as well as associations with therapeutic effectiveness. Although the association between neurotransmitters found in the CNS and urine is largely unknown, much clinical data exists that suggests urinary neurotransmitter testing is a powerful tool to assess nervous system function and thereby allow physicians to monitor and treat various clinical conditions.

Acknowledgments

We would like to thank Chasatie Tretsven and Diana Colton for developing the graphics, Paul Wynveen, Dr. Han Huisman, and Dr. Mikaela Nichkova for proofreading, and Dr. Gottfried Kellermann for his encouragement and support for this manuscript.

References

- Acara, M., Rennick, B., 1973. Regulation of plasma choline by the renal tubule: bidirectional transport of choline. *Am. J. Physiol.* 225, 1123–1128.
- Amara, S.G., Kuhar, M.J., 1993. Neurotransmitter transporters: recent progress. *Annu. Rev. Neurosci.* 16, 73–93.
- Anderson, G.M., Dover, M.A., Yang, B.P., Holahan, J.M., Shaywitz, S.E., Marchione, K.E., Hall, L.M., Fletcher, J.M., Shaywitz, B.A., 2000. Adrenomedullary function during cognitive testing in attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 39, 635–643.
- Arriza, J.L., Fairman, W.A., Wadiche, J.L., Murdoch, G.H., Kavanaugh, M.P., Amara, S.G., 1994. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J. Neurosci.* 14, 5559–5569.
- Asano, K., Lilly, C.M., O'Donnell, W.J., Israel, E., Fischer, A., Ransil, B.J., Drazen, J.M., 1995. Diurnal variation of urinary leukotriene E4 and histamine excretion rates in normal subjects and patients with mild-to-moderate asthma. *J. Allergy Clin. Immunol.* 96, 643–651.
- Baker, G.B., Bornstein, R.A., Rouget, A.C., Ashton, S.E., van Muyden, J.C., Coutts, R.T., 1991. Phenylethylaminergic mechanisms in attention-deficit disorder. *Biol. Psychiatry* 29, 15–22.
- Ballabh, P., Braun, A., Nedergaard, M., 2004. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol. Dis.* 16, 1–13.
- Benazzi, F., 2006. Various forms of depression. *Dialogues Clin. Neurosci.* 8, 151–161.
- Berry, M.D., 2004. Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. *J. Neurochem.* 90, 257–271.
- Bloom, F.E., Kupfer, D.J., 1995. Psychopharmacology: the fourth generation of progress, pp. 407–471.
- Boucher, W., Stern, J.M., Kotsinyan, V., Kempuraj, D., Papaliodis, D., Cohen, M.S., Theoharides, T.C., 2008. Intravesical nanocrystalline silver decreases experimental bladder inflammation. *J. Urol.* 179, 1598–1602.
- Breidert, T., Spitzenberger, F., Grundemann, D., Schomig, E., 1998. Catecholamine transport by the organic cation transporter type 1 (OCT1). *Br. J. Pharmacol.* 125, 218–224.
- Busch, A.E., Karbach, U., Miska, D., Gorboulev, V., Akhoundova, A., Volk, C., Arndt, P., Ulzheimer, J.C., Sonders, M.S., Baumann, C., Waldegger, S., Lang, F., Koepsell, H., 1998. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol. Pharmacol.* 54, 342–352.
- Busch, A.E., Quester, S., Ulzheimer, J.C., Gorboulev, V., Akhoundova, A., Waldegger, S., Lang, F., Koepsell, H., 1996. Monoamine neurotransmitter transport mediated by the polyspecific cation transporter rOCT1. *FEBS Lett.* 395, 153–156.
- Castellanos, F.X., Elia, J., Kruesi, M.J., Gulotta, C.S., Mefford, I.N., Potter, W.Z., Ritchie, G.F., Rapoport, J.L., 1994. Cerebrospinal fluid monoamine metabolites in boys with attention-deficit hyperactivity disorder. *Psychiatry Res.* 52, 305–316.
- Chekhonin, V.P., Baklaushv, V.P., Kogan, B.M., Savchenko, E.A., Lebedev, S.V., Man'kovskaya, I.V., Filatova, T.S., Yusupova, I.U., Dmitrieva, T.B., 2000. Catecholamines and their metabolites in the brain and urine of rats with experimental Parkinson's disease. *Bull. Exp. Biol. Med.* 130, 805–809.
- Chen, N.H., Reith, M.E., Quick, M.W., 2004. Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. *Pflugers Arch.* 447, 519–531.
- Cook, I.A., 2008. Biomarkers in psychiatry: potentials, pitfalls, and pragmatics. *Prim. Psychiatry* 15, 54–59.
- Cormier, E., 2008. Attention deficit/hyperactivity disorder: a review and update. *J. Pediatr. Nurs.* 23, 345–357.
- Dantonello, T.M., Kuster, E., Muhlbaier, B., 1998. Urinary dopamine and renal handling of L-DOPA in fasted spontaneously hypertensive rats. *Kidney Blood Press. Res.* 21 (6), 438–444.
- Davis, T.P., Gehrke, C.W., Gehrke Jr., C.W., Cunningham, T.D., Kuo, K.C., Gerhardt, K.O., Johnson, H.D., Williams, C.H., 1978. High-performance liquid-chromatographic separation and fluorescence measurement of biogenic amines in plasma, urine, and tissue. *Clin. Chem.* 24, 1317–1324.
- Delahanty, D.L., Nugent, N.R., Christopher, N.C., Walsh, M., 2005. Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims. *Psychoneuroendocrinology* 30, 121–128.
- Duncan, M.W., Compton, P., Lazarus, L., Smythe, G.A., 1988. Measurement of norepinephrine and 3,4-dihydroxyphenylglycol in urine and plasma for the diagnosis of pheochromocytoma. *N. Engl. J. Med.* 319, 136–142.
- Eisenhofer, G., Kopin, I.J., Goldstein, D.S., 2004. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol. Rev.* 56, 331–349.
- Eisenhofer, G., McCarty, R., Pacak, K., Russ, H., Schomig, E., 1996. Disprocyntium24, a novel inhibitor of the extraneuronal monoamine transporter, has potent effects on the inactivation of circulating noradrenaline and adrenaline in conscious rat. *Naunyn Schmiedebergs Arch. Pharmacol.* 354, 287–294.

- Elenkov, I.J., Chrousos, G.P., 2002. Stress hormones, proinflammatory and anti-inflammatory cytokines, and autoimmunity. *Ann. N. Y. Acad. Sci.* 966, 290–303.
- Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2009. The sympathetic nerve – An integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.* 52, 595–638.
- Elia, J., Borcherding, B.G., Potter, W.Z., Mefford, I.N., Rapoport, J.L., Keysor, C.S., 1990. Stimulant drug treatment of hyperactivity: biochemical correlates. *Clin. Pharmacol. Ther.* 48, 57–66.
- Engel, A., von Euler, U.S., 1950. Diagnostic value of increased urinary output of pheochromocytoma. *Lancet* 2, 387.
- Engel, K., Wang, J., 2005. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Mol. Pharmacol.* 68, 1397–1407.
- Fernstrom, J.D., Fernstrom, M.H., 2007. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J. Nutr.* 137, 1539S–1547S.
- Francis, R.C., Pickerodt, P.A., Salewski, L., Boemke, W., Hohne, C., 2010. Detection of catecholamines and metanephrines by radio-immunoassay in canine plasma. *Vet. J.* 183, 228–231.
- Gallowitsch-Puerta, M., Pavlov, V.A., 2007. Neuro-immune interactions via the cholinergic anti-inflammatory pathway. *Life Sci.* 80, 2325–2329.
- Gorboulev, V., Ulzheimer, J.C., Akhoundova, A., Ulzheimer-Teuber, I., Karbach, U., Quester, S., Baumann, C., Lang, F., Busch, A.E., Koepsell, H., 1997. Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol.* 16, 871–881.
- Graefe, K.H., Friedgen, B., Wolfel, R., Bossle, F., Russ, H., Schomig, E., 1997. 1,1'-Diisopropyl-2,4'-cyanine (disprocymin24), a potent uptake2 blocker, inhibits the renal excretion of catecholamines. *Naunyn Schmiedeberg's Arch. Pharmacol.* 356, 115–125.
- Grossman, F., Potter, W.Z., 1999. Catecholamines in depression: a cumulative study of urinary norepinephrine and its major metabolites in unipolar and bipolar depressed patients versus healthy volunteers at the NIMH. *Psychiatry Res.* 87, 21–27.
- Grundemann, D., Babin-Ebell, J., Martel, F., Ording, N., Schmidt, A., Schomig, E., 1997. Primary structure and functional expression of the apical organic cation transporter from kidney epithelial LLC-PK1 cells. *J. Biol. Chem.* 272, 10408–10413.
- Grundemann, D., Koster, S., Kiefer, N., Breidert, T., Engelhardt, M., Spitzenberger, F., Obermuller, N., Schomig, E., 1998. Transport of monoamine transmitters by the organic cation transporter type 2, OCT2. *J. Biol. Chem.* 273, 30915–30920.
- Hanna, G.L., Ornitz, E.M., Hariharan, M., 1996. Urinary catecholamine excretion and behavioral differences in ADHD and normal boys. *J. Child Adolesc. Psychopharmacol.* 6, 63–73.
- Hardebo, J.E., Owman, C., 1980. Characterization of the in vitro uptake of monoamines into brain microvessels. *Acta Physiol. Scand.* 108, 223–229.
- Hawkins, R.A., O'Kane, R.L., Simpson, I.A., Vina, J.R., 2006. Structure of the blood-brain barrier and its role in the transport of amino acids. *J. Nutr.* 136, 218S–226S.
- Hayer-Zillgen, M., Bruss, M., Bonisch, H., 2002. Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. *Br. J. Pharmacol.* 136, 829–836.
- Henderson, J., 2005. Ernest starling and 'Hormones': an historical commentary. *J. Endocrinol.* 184, 5–10.
- Hermens, D.F., Rowe, D.L., Gordon, E., Williams, L.M., 2006. Integrative neuroscience approach to predict ADHD stimulant response. *Expert. Rev. Neurother.* 6, 753–763.
- Hershko, A.Y., Dranitzki, Z., Ulmanski, R., Levi-Schaffer, F., Naparstek, Y., 2001. Constitutive hyperhistaminaemia: a possible mechanism for recurrent anaphylaxis. *Scand. J. Clin. Lab. Invest.* 61, 449–452.
- Hogan, A.D., Schwartz, L.B., 1997. Markers of mast cell degranulation. *Methods* 13, 43–52.
- Holsboer, F., 2008. How can we realize the promise of personalized antidepressant medicines? *Nat. Rev. Neurosci.* 9, 638–646.
- Hosoya, K., Sugawara, M., Asaba, H., Terasaki, T., 1999. Blood-brain barrier produces significant efflux of L-aspartic acid but not D-aspartic acid: in vivo evidence using the brain efflux index method. *J. Neurochem.* 73, 1206–1211.
- Hubner, C., Wedding, U., Strater, J., Limberg, B., Stremmel, W., 1997. Clinical stable systemic mastocytosis with interferon alpha-2b therapy. *J. Intern. Med.* 241, 529–533.
- Hughes, J.W., Watkins, L., Blumenthal, J.A., Kuhn, C., Sherwood, A., 2004. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J. Psychosom. Res.* 57, 353–358.
- Huisman, H., Wynveen, P., Setter, P.W., 2010. Studies on the immune response and preparation of antibodies against a large panel of conjugated neurotransmitters and biogenic amines: specific polyclonal antibody response and tolerance. *J. Neurochem.* 112, 829–841.
- Husztai, Z., Deli, M.A., Joo, F., 1995. Carrier-mediated uptake and release of histamine by cultured rat cerebral endothelial cells. *Neurosci. Lett.* 184, 185–188.
- Joyce, P.R., Fergusson, D.M., Woollard, G., Abbott, R.M., Horwood, L.J., Upton, J., 1995. Urinary catecholamines and plasma hormones predict mood state in rapid cycling bipolar affective disorder. *J. Affect. Disord.* 33, 233–243.
- Kagedal, B., Goldstein, D.S., 1988. Catecholamines and their metabolites. *J. Chromatogr.* 429, 177–233.
- Kakee, A., Takanaga, H., Terasaki, T., Naito, M., Tsuruo, T., Sugiyama, Y., 2001. Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier. *J. Neurochem.* 79, 110–118.
- Karbach, U., Kricke, J., Meyer-Wentrup, F., Gorboulev, V., Volk, C., Loffing-Cueni, D., Kaissling, B., Bachmann, S., Koepsell, H., 2000. Localization of organic cation transporters OCT1 and OCT2 in rat kidney. *Am. J. Physiol. Renal Physiol.* 279, F679–F687.
- Keckuda, R., Prasad, P.D., Wu, X., Wang, H., Fei, Y.J., Leibach, F.H., Ganapathy, V., 1998. Cloning and functional characterization of a potential-sensitive, polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. *J. Biol. Chem.* 273, 15971–15979.
- Koepsell, H., Busch, A., Gorboulev, V., Arndt, P., 1998. Structure and function of renal organic cation transporters. *News Physiol. Sci.* 13, 11–16.
- Koepsell, H., Gorboulev, V., Arndt, P., 1999. Molecular pharmacology of organic cation transporters in kidney. *J. Membr. Biol.* 167, 103–117.
- Kopp, U., Bradley, T., Hjemedahl, P., 1983. Renal venous outflow and urinary excretion of norepinephrine, epinephrine, and dopamine during graded renal nerve stimulation. *Am. J. Physiol.* 244, E52–E60.
- Koslow, S.H., Maas, J.W., Bowden, C.L., Davis, J.M., Hanin, I., Javaid, J., 1983. CSF and urinary biogenic amines and metabolites in depression and mania. A controlled, univariate analysis. *Arch. Gen. Psychiatry* 40, 999–1010.
- Koslow, S.H., Maas, J.W., Bowden, C.L., Davis, J.M., Hanin, I., Javaid, J., 1986. Tricyclic antidepressant washout effects on cerebrospinal fluid and urinary monoamine and metabolites. *Arch. Gen. Psychiatry* 43, 1012–1013.
- Kotzailias, N., Marker, M., Jilma, B., 2004. Early effects of paroxetine on serotonin storage, plasma levels, and urinary excretion: a randomized, double-blind, placebo-controlled trial. *J. Clin. Psychopharmacol.* 24, 536–539.
- Kudva, Y.C., Sawka, A.M., Young Jr., W.F., 2003. Clinical review 164: the laboratory diagnosis of adrenal pheochromocytoma: the Mayo Clinic experience. *J. Clin. Endocrinol. Metab.* 88, 4533–4539.
- Kusaga, A., Yamashita, Y., Koeda, T., Hiratani, M., Kaneko, M., Yamada, S., Matsushita, T., 2002. Increased urine phenylethylamine after methylphenidate treatment in children with ADHD. *Ann. Neurol.* 52, 372–374.
- Lamale, L.M., Lutgendorf, S.K., Zimmerman, M.B., Kreder, K.J., 2006. Interleukin-6, histamine, and methylhistamine as diagnostic markers for interstitial cystitis. *Urology* 68, 702–706.
- Lechin, F., van der Dijs, D.B., 2006. Central nervous system circuitry and peripheral neural sympathetic activity responsible for essential hypertension. *Curr. Neurovasc. Res.* 3, 307–325.
- Lechin, F., van der Dijs, D.B., Lechin, M.E., 1996. Plasma neurotransmitters and functional illness. *Psychother. Psychosom.* 65, 293–318.
- Le-Niculescu, H., Kurian, S.M., Yehyaw, N., Dike, C., Patel, S.D., Edenberg, H.J., Tsuang, M.T., Salomon, D.R., Nurnberger Jr., J.I., Niculescu, A.B., 2008. Identifying blood biomarkers for mood disorders using convergent functional genomics. *Mol. Psychiatry* 14, 156–174.
- Lepschy, M., Rettenbacher, S., Touma, C., Palme, R.G., 2008. Excretion of catecholamines in rats, mice and chicken. *J. Comp. Physiol. [B]* 178, 629–636.
- Lynn-Bullock, C.P., Welshhans, K., Pallas, S.L., Katz, P.S., 2004. The effect of oral 5-HTP administration on 5-HTP and 5-HT immunoreactivity in monoaminergic brain regions of rats. *J. Chem. Neuroanat.* 27, 129–138.
- Maas, J.W., Koslow, S.H., Davis, J., Katz, M., Frazer, A., Bowden, C.L., Berman, N., Gibbons, R., Stokes, P., Landis, D.H., 1987. Catecholamine metabolism and disposition in healthy and depressed subjects. *Arch. Gen. Psychiatry* 44, 337–344.
- Maas, J.W., Landis, D.H., 1965. A technique for assaying the kinetics of norepinephrine metabolism in the central nervous system in vivo. *Psychosom. Med.* 28, 247–256.
- Manyam, B.V., Hare, T.A., 1983. Cerebrospinal fluid GABA measurements: basic and clinical considerations. *Clin. Neuropharmacol.* 6, 25–36.
- Matsuda, F., Hayashi, T., Naruse, H., 1991. A study on the origins of urinary serotonin and tryptamine. *Tokai J. Exp. Clin. Med.* 16, 245–252.
- Mayer, E.A., Tillisch, K., Bradesi, S., 2006. Review article: modulation of the brain-gut axis as a therapeutic approach in gastrointestinal disease. *Aliment. Pharmacol. Ther.* 24, 919–933.
- Moleman, P., Tulen, J.H., Blankstijn, P.J., Man in't Veld, A.J., Boomsma, F., 1992. Urinary excretion of catecholamines and their metabolites in relation to circulating catecholamines. Six-hour infusion of epinephrine and norepinephrine in healthy volunteers. *Arch. Gen. Psychiatry* 49, 568–572.
- Mooney, J.J., Cole, J.O., Schatzberg, A.F., Gerson, B., Schildkraut, J.J., 1985. Pre-treatment urinary MHPG levels as predictors of antidepressant responses to alprazolam. *Am. J. Psychiatry* 142, 366–367.
- Mooney, J.J., Samson, J.A., Hennen, J., Pappalardo, K., McHale, N., Alpert, J., Koutsos, M., Schildkraut, J.J., 2008. Enhanced norepinephrine output during long-term desipramine treatment: a possible role for the extraneuronal monoamine transporter (SLC22A3). *J. Psychiatr. Res.* 42, 605–611.
- Mooney, J.J., Schatzberg, A.F., Cole, J.O., Kizuka, P.P., Salomon, M., Lerbinger, J., Pappalardo, K.M., Gerson, B., Schildkraut, J.J., 1988. Rapid antidepressant response to alprazolam in depressed patients with high catecholamine output and heterologous desensitization of platelet adenylate cyclase. *Biol. Psychiatry* 23, 543–559.
- Mooney, J.J., Schatzberg, A.F., Cole, J.O., Samson, J.A., Waternaux, C., Gerson, B., Pappalardo, K.M., Schildkraut, J.J., 1991. Urinary 3-methoxy-4-hydroxyphenylglycol and the depression-type score as predictors of differential responses to antidepressants. *J. Clin. Psychopharmacol.* 11, 339–343.
- Moyer, T.P., Jiang, N.S., Tyce, G.M., Sheps, S.G., 1979. Analysis for urinary catecholamines by liquid chromatography with amperometric detection: methodology and clinical interpretation of results. *Clin. Chem.* 25, 256–263.
- Murdoch, R.D., Youtlen, L.J., Williams, A.J., Howland, K., 1993. Plasma concentrations and urinary excretion of histamine after inhalation and subcutaneous injection of histamine. *Br. J. Clin. Pharmacol.* 35, 171–177.
- Myers, G., Donlon, M., Kaliner, M., 1981. Measurement of urinary histamine: development of methodology and normal values. *J. Allergy Clin. Immunol.* 67, 305–311.

- Nakatani, Y., Sato-Suzuki, I., Tsujino, N., Nakasato, A., Seki, Y., Fumoto, M., Arita, H., 2008. Augmented brain 5-HT crosses the blood-brain barrier through the 5-HT transporter in rat. *Eur. J. Neurosci.* 27, 2466–2472.
- Nemeroff, C.B., 2007. The burden of severe depression: a review of diagnostic challenges and treatment alternatives. *J. Psychiatr. Res.* 41, 189–206.
- Nigrovic, L.E., McQueen, A.A., Neuman, M.I., 2007. Lumbar puncture success rate is not influenced by family-member presence. *Pediatrics* 120, e777–e782.
- Nishiwaki, F., Kuroda, K., Inoue, Y., Endo, G., 2000. Determination of histamine, 1-methylhistamine and N-methylhistamine by capillary electrophoresis with micelles. *Biomed. Chromatogr.* 14, 184–187.
- Noshiro, T., Saigusa, T., Way, D., Dorward, P.K., McGrath, B.P., 1991. Norepinephrine spillover faithfully reflects renal sympathetic nerve activity in conscious rabbits. *Am. J. Physiol.* 261, F44–F50.
- Ohtsuki, S., 2004. New aspects of the blood-brain barrier transporters; its physiological roles in the central nervous system. *Biol. Pharm. Bull.* 27, 1489–1496.
- Okumura, T., Nakajima, Y., Matsuoka, M., Takamatsu, T., 1997. Study of salivary catecholamines using fully automated column-switching high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* 694, 305–316.
- Otte, C., Neylan, T.C., Pipkin, S.S., Browner, W.S., Whooley, M.A., 2005. Depressive symptoms and 24-hour urinary norepinephrine excretion levels in patients with coronary disease: findings from the Heart and Soul Study. *Am. J. Psychiatry* 162, 2139–2145.
- Panholzer, T.J., Beyer, J., Lichtwald, K., 1999. Coupled-column liquid chromatographic analysis of catecholamines, serotonin, and metabolites in human urine. *Clin. Chem.* 45, 262–268.
- Pardridge, W.M., 1999. Blood-brain barrier biology and methodology. *J. Neurovirol.* 5, 556–569.
- Pliszka, S.R., Maas, J.W., Javors, M.A., Rogeness, G.A., Baker, J., 1994. Urinary catecholamines in attention-deficit hyperactivity disorder with and without comorbid anxiety. *J. Am. Acad. Child Adolesc. Psychiatry* 33, 1165–1173.
- Rosano, T.G., Swift, T.A., Hayes, L.W., 1991. Advances in catecholamine and metabolite measurements for diagnosis of pheochromocytoma. *Clin. Chem.* 37, 1854–1867.
- Roy, A., Linnoila, M., Karoum, F., Pickar, D., 1986a. Relative activity of metabolic pathways for norepinephrine in endogenous depression. *Acta Psychiatr. Scand.* 73, 624–628.
- Roy, A., Pickar, D., De, J.J., Karoum, F., Linnoila, M., 1988. Norepinephrine and its metabolites in cerebrospinal fluid, plasma, and urine. Relationship to hypothalamic-pituitary-adrenal axis function in depression. *Arch. Gen. Psychiatry* 45, 849–857.
- Roy, A., Pickar, D., Douillet, P., Karoum, F., Linnoila, M., 1986b. Urinary monoamines and monoamine metabolites in subtypes of unipolar depressive disorder and normal controls. *Psychol. Med.* 16, 541–546.
- Roy, A., Pollack, S., 1994. Are cerebrospinal fluid or urinary monoamine metabolite measures stronger correlates of suicidal behavior in depression? *Neuropsychobiology* 29, 164–167.
- Rubin, L.L., Staddon, J.M., 1999. The cell biology of the blood-brain barrier. *Annu. Rev. Neurosci.* 22, 11–28.
- Schatzberg, A.F., 1998. Noradrenergic versus serotonergic antidepressants: predictors of treatment response. *J. Clin. Psychiatry* 59 (Suppl. 14), 15–18.
- Schildkraut, J.J., Orsulak, P.J., Schatzberg, A.F., Gudeman, J.E., Cole, J.O., Rohde, W.A., LaBrie, R.A., 1978. Toward a biochemical classification of depressive disorders. I. Differences in urinary excretion of MHPG and other catecholamine metabolites in clinically defined subtypes of depressions. *Arch. Gen. Psychiatry* 35, 1427–1433.
- Schildkraut, J.J., Schatzberg, A.F., Samson, J.A., Rosenbaum, A., Bowden, C.L., 1992. Norepinephrine output and metabolism in depressed patients during antidepressant treatments. *Clin. Neuropharmacol.* 15 (Suppl. 1 Pt A), 323A–324A.
- Schwartz, L.B., 2004. Effector cells of anaphylaxis: mast cells and basophils. *Novartis Found. Symp.* 257, 65–74.
- Schwarz, E., Bahn, S., 2008. The utility of biomarker discovery approaches for the detection of disease mechanisms in psychiatric disorders. *Br. J. Pharmacol.* 153 (Suppl. 1), S133–S136.
- Seegal, R.F., Brosch, K.O., Bush, B., 1986. High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. *J. Chromatogr.* 377, 131–144.
- Skoner, D.P., Gentile, D.A., Fireman, P., Cordero, K., Doyle, W.J., 2001. Urinary histamine metabolite elevations during experimental influenza infection. *Ann. Allergy Asthma Immunol.* 87, 303–306.
- Swann, A.C., Koslow, S.H., Katz, M.M., Maas, J.W., Javadi, J., Secunda, S.K., Robins, E., 1987. Lithium carbonate treatment of mania. Cerebrospinal fluid and urinary monoamine metabolites and treatment outcome. *Arch. Gen. Psychiatry* 44, 345–354.
- Szabo, A., Billett, E., Turner, J., 2001. Phenylethylamine, a possible link to the antidepressant effects of exercise? *Br. J. Sports Med.* 35, 342–343.
- Takanaga, H., Ohtsuki, S., Hosoya, K., Terasaki, T., 2001. GAT2/BGT-1 as a system responsible for the transport of gamma-aminobutyric acid at the mouse blood-brain barrier. *J. Cereb. Blood Flow Metab.* 21, 1232–1239.
- Takasaka, Y., Tsukasaki, K., Jubashi, T., Tomonaga, M., Kamihira, S., Makiyama, K., 1998. Systemic mastocytosis with extensive polypoid lesions in the intestines; successful treatment with interferon-alpha. *Intern. Med.* 37, 484–488.
- Tamai, I., Tsuji, A., 2000. Transporter-mediated permeation of drugs across the blood-brain barrier. *J. Pharm. Sci.* 89, 1371–1388.
- Tang, A.W., 2003. A practical guide to anaphylaxis. *Am. Fam. Physician* 68, 1325–1332.
- von Euler, U.S., Hellner, S., 1951. Excretion of noradrenaline, adrenaline, and hydroxytyramine in urine. *Acta Physiol. Scand.* 22, 160–167.
- von Euler, U.S., Hellner-Bjorkman, S., Orwen, I., 1955. Diurnal variations in the excretion of free and conjugated noradrenaline and adrenaline in urine from healthy subjects. *Acta Physiol. Scand. Suppl.* 33, 10–16.
- von Euler, U.S., Luft, R., 1951. Noradrenaline output in urine after infusion in man. *Br. J. Pharmacol. Chemother.* 6, 286–288.
- Wa, T.C., Burns, N.J., Williams, B.C., Freestone, S., Lee, M.R., 1995. Blood and urine 5-hydroxytryptophan and 5-hydroxytryptamine levels after administration of two 5-hydroxytryptamine precursors in normal man. *Br. J. Clin. Pharmacol.* 39, 327–329.
- Wakayama, K., Ohtsuki, S., Takanaga, H., Hosoya, K., Terasaki, T., 2002. Localization of norepinephrine and serotonin transporter in mouse brain capillary endothelial cells. *Neurosci. Res.* 44, 173–180.
- Westermann, J., Hubl, W., Kaiser, N., Salewski, L., 2002. Simple, rapid and sensitive determination of epinephrine and norepinephrine in urine and plasma by non-competitive enzyme immunoassay, compared with HPLC method. *Clin. Lab.* 48, 61–71.
- Westphal, S.A., 2005. Diagnosis of a pheochromocytoma. *Am. J. Med. Sci.* 329, 18–21.
- Wong, D.F., Potter, W.Z., Brasic, J.R., 2002. Proof of concept: functional models for drug development in humans. In: Davis, K.L., Charney, D., Coyle, J.T., Nemeroff, C. (Eds.), *Neuropsychopharmacology: Fifth Generation of progress*. Lippincott Williams & Wilkins, Philadelphia, pp. 457–473.
- Wood, J.H., 1980. Neurochemical analysis of cerebrospinal fluid. *Neurology* 30, 645–651.
- Wu, X., Huang, W., Ganapathy, M.E., Wang, H., Kekuda, R., Conway, S.J., Leibach, F.H., Ganapathy, V., 2000. Structure, function, and regional distribution of the organic cation transporter OCT3 in the kidney. *Am. J. Physiol. Renal Physiol.* 279, F449–F458.
- Wu, X., Kekuda, R., Huang, W., Fei, Y.J., Leibach, F.H., Chen, J., Conway, S.J., Ganapathy, V., 1998. Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. *J. Biol. Chem.* 273, 32776–32786.
- Wurtman, R.J., 1987. Nutrients affecting brain composition and behavior. *Integr. Psychiatry* 5, 226–238.
- Wurtman, 1988. Effects of dietary amino acids, carbohydrates, and choline on neurotransmitter synthesis. *Mt. Sinai J. Med.* 55, 75–86.
- Yabuuchi, H., Tamai, I., Nezu, J., Sakamoto, K., Oku, A., Shimane, M., Sai, Y., Tsuji, A., 1999. Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J. Pharmacol. Exp. Ther.* 289, 768–773.
- Yamatodani, A., 1990. [Plasma and urinary histamine assay and the clinical significance]. *Nippon Rinsho* 48 (Suppl.), 171–174.
- Zametkin, A.J., Hamburger, S.D., 1988. The effect of methylphenidate on urinary catecholamine excretion in hyperactivity: a partial replication. *Biol. Psychiatry* 23, 350–356.
- Zametkin, A.J., Karoum, F., Linnoila, M., Rapoport, J.L., Brown, G.L., Chuang, L.W., Wyatt, R.J., 1985. Stimulants, urinary catecholamines, and indoleamines in hyperactivity. A comparison of methylphenidate and dextroamphetamine. *Arch. Gen. Psychiatry* 42, 251–255.
- Zarate, A., Saucedo, R., 2005. On the centennial of hormones. A tribute to Ernest H. Starling and William M. Bayliss. *Gac. Med. Mex.* 141, 437–439.