Homovanillic Acid Measurement in Clinical Research: A Review of Methodology

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Abstract

Despite its serious limitations, measuring the concentrations of homovanillic acid (HVA) in body fluids is perhaps the most direct method currently available to assess the changing activity of central dopamine (DA) neurons in living humans. The concentrations of HVA in body fluids are determined not only by the activity of central DA neurons but also by a number of unrelated factors such as the other sources of HVA and the elimination of HVA from the body. This article reviews many factors that help to determine the concentrations of HVA in body fluids and therefore must be considered in studies employing HVA as a possible index of central DA neuronal activity. The need for further methodological work is indicated to improve the use of body fluid HVA measurements in clinical research.

Dysfunctions of brain dopamine (DA) neurons are implicated in many common disorders such as schizophrenia, other psychotic disorders, and Parkinson's disease. Studying the brain's DA neuronal activity (neuronal firing rates or nerve impulse traffic) may elucidate the psychopathology of these disorders. In humans, the measurement of major DA metabolite, homovanillic acid (HVA), in easily accessible body fluids (i.e., cerebrospinal fluid [CSF], blood, and urine) has been used to estimate brain DA neuronal production of HVA and possibly brain DA neuronal activity. This approach is based on the assumption that metabolite production by neurons reflects neurotransmitter release and metabolism and therefore neuronal activity (see section, "HVA Measurement as Possible Indicator of Central DA Neuronal Activity"). In human studies, HVA is measured in disease states or in experimental conditions (e.g., drug treatment) and compared with appropriate controls to make inferences regarding the activity of brain DA neurons. Doing so provides clues to the role of brain DA neurons in the pathophysiology of the disease under study and helps explain the mechanism of action of the drugs influencing DA neuronal activity.

The body fluids in which HVA concentrations are measured (CSF, blood, and urine) are far removed from the locations of brain DA neurons; therefore, many unrelated factors can confound the HVA measurements. For example, HVA is also produced by sources other than brain DA neurons, such as norepinephrine (NE) neurons (see section, "Sources of HVA"). Factors affecting the movement of HVA from the sites of production to the sites of measurement and its elimination from the body can also affect HVA measurements. This review attempts to briefly discuss and summarize factors that need to be considered in clinical studies using HVA as a possible index of central DA neuronal activity. It is hoped that this review will be helpful in interpreting previously collected data and will provide rationale and impetus for further research in improving the methodology of employing HVA as a possible index of central DA neuronal activity.

DA Metabolism and Production of HVA

Several recent reviews of DA metabolism and HVA formation are avail-
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Simplified steps in the formation and metabolism of catecholamines (i.e., DA, NE, and epinephrine [EPI]) are shown in figure 1. DA synthesis begins with the amino acid tyrosine. Tyrosine is normally present in the circulation and is derived either from food intake or from protein breakdown. An active transport mechanism is responsible for concentrating tyrosine in catecholamine neurons. Several other amino acids share the same transport mechanism. Thus, the uptake of tyrosine can be influenced by the relative concentrations of other amino acids (Cooper et al. 1986). Once inside the cell, tyrosine is converted into dihydroxyphenylalanine (DOPA) by the action of tyrosine hydroxylase. DOPA is then converted into DA by DOPA-decarboxylase (also known as aromatic amino acid decarboxylase). In the case of DA neurons, DA is taken up in the storage vesicles, stored as such, and released as a neurotransmitter. In noradrenergic neurons, however, DA is also taken up by the storage vesicles but is further converted inside the storage vesicle into NE by dopamine-beta-hydroxylase.

The conversion of tyrosine into DOPA is generally the rate-limiting step in the synthesis of DA. Under physiological conditions, impulse flow in DA neurons is the main factor that regulates the formation of DA and ultimately HVA. Other factors (e.g., presynaptic autoreceptors, tyrosine availability, cellular DA and cofactor concentrations, and the degree of activation of tyrosine hydroxylase) can also influence the rate of synthesis of DA and hence the formation of HVA (Goldstein and Greene 1987; Roth et al. 1987).

The DA levels in the nerve terminals are generally remarkably constant during varying degrees of DA neuronal activity (Roth et al. 1978), suggesting a tight regulation of DA synthesis, release, and metabolism (Cooper et al. 1986).

When an impulse arrives at a DA nerve terminal, DA is released into the synaptic cleft. The released DA interacts with DA receptors present on both sides of the synaptic cleft. This action of DA is terminated mainly by an active reuptake of the released DA back into the DA nerve terminal. Reabsorbed DA is metabolized into 3,4-dihydroxyphenylacetic acid (DOPAC) by the action of monoamine oxidase (MAO). This metabolic step is believed to be intraneuronal (Westerink 1985; Wood and Altar 1988). Much of the remaining DA (approximately 20%) left in the synaptic cleft is quickly metabolized into 3-methoxytyramine (3-MT) by the action of catechol-O-methyl transferase (COMT). This step is believed to be extraneuronal (Westerink 1985; Wood and Altar 1988). COMT is located extraneuronally on the synaptic surfaces of the cell membranes. Both 3-MT and DOPAC are then acted on by MAO and COMT enzymes, respectively, and are converted into HVA, which is the secondary and final metabolite of DA. Unlike rats, in which DOPAC is a major metabolite of DA, humans convert most of the DOPAC into HVA, which is the predominant human metabolite of DA. The extraneuronal metabolite 3-MT, measured locally, is believed to be a better index of DA release per se, whereas DOPAC, the intraneuronal metabolite, is regarded as a combined measure of DA release as well as of DA metabolism. Since most of the HVA is derived via DOPAC, HVA

**Figure 1. Simplified sequence of catecholamine metabolism**

![Figure 1](image_url)

**Legend**: 
- Tyrosine
- DOPA
- DA
- NE
- Epinephrine
- MAO
- COMT
- PST
- DOPAC
- 3-MT
- HVA
- MHPG
- VMA
- DA-Sulfate

**Key**: 
- DOPA = dihydroxyphenylalanine; DA = dopamine; NE = norepinephrine; DOPAC = 3,4-dihydroxyphenylacetic acid; 3-MT = 3-methoxytyramine; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol; VMA = vanillylmandelic acid; MAO = monoamine oxidase; COMT = catechol-O-methyl transferase; PST = phenol-sulfo transferase.
is also regarded as a combined measure of DA release as well as of its intraneuronal synthesis and metabolism (Westerink 1985; Roth et al. 1987; Wood and Altar 1988).

In addition to being metabolized into DOPAC, 3-MT, and HVA, DA is also conjugated in the brain, mostly to sulfate by the action of phenol-sulfo transferase (PST). Although DA can be sulfated to a significant degree in rats, sulfate conjugation in the human brain probably occurs only to a minor extent. Significant sulfate conjugation does occur outside the brain in humans, but it is mostly confined to the gastrointestinal tract for ingested DA. This point is evidenced by the finding that after foods containing DOPA or DA are ingested, large amounts of DA sulfates are found in plasma (Davidson et al. 1981) and in urine, but more than two-thirds of ingested DOPA is still converted into deaminated metabolites, namely, DOPAC and HVA (Sandler 1972). Ingested DOPA has been shown to result in up to a 100-fold increase in plasma DA sulfate (Cuche et al. 1985). But, after labeled DA is administered intravenously, less than 2 percent was recovered as a conjugate in urine (Goodall and Alton 1968).

Conjugation (mostly to sulfate) of DA metabolites (e.g., DOPAC and HVA) also occurs in some species. For example, significant amounts of DA metabolites are found as conjugates in rat brain and body fluids. Therefore, measurements of conjugates of DA metabolites are critical in obtaining a complete picture of metabolite changes in rats (Cottet-Emard and Peyrin 1982; Sarna et al. 1984; Curzon et al. 1985; Tavitian et al. 1986). However, in primates (including humans) conjugation does not appear to occur significantly in the brain (Bacopoulos et al. 1978b), and only very small amounts of HVA are found as conjugates in human body fluids such as plasma (Garcia et al. 1989) and urine (Buneau et al. 1986; Barthelemy and Buneau 1988; Tuchman and Stoeckeler 1988). In humans, the significance of conjugates of HVA in body fluids for the study of central DA turnover is unclear.

In summary, endogenously produced DA is predominantly metabolized to HVA in humans and mostly excreted in a free unconjugated form. Ingested DA is partly conjugated in the intestine and the liver and is excreted as DA sulfate in urine. The rest of ingested DA is metabolized to HVA that is mostly excreted in an unconjugated form.

**HVA Measurement as Possible Indicator of Central DA Neuronal Activity**

An estimation of brain DA neuronal activity from the metabolite production rate is based on the assumption that nerve impulse traffic, DA release, DA synthesis, and metabolism are all linked. However, these linked relationships are complex, are regulated by poorly understood mechanisms, and may not hold true under all circumstances. For example, DA release and metabolism have been suggested to occur in rat substantia nigra beyond the control of nerve impulses (Nissbrandt et al. 1985). In addition, mechanical and pharmacological interruption of impulse flow in rat nigrostriatal DA neurons can result in a paradoxical increase in DA synthesis (Roth et al. 1978). Thus, caution must be exercised in drawing inferences regarding brain DA neuronal activity from changes in the metabolite concentrations.

In most cases, however, DA metabolite concentrations measured locally in brain tissue are regarded as a reasonable combined measure of DA release, synthesis, and metabolism and therefore of impulse flow in brain DA neurons. This is supported by animal studies. For example, increasing the impulse flow in DA pathways in rats correlates with an increased concentration of DA metabolites in the innervated regions of the brain (Bunney et al. 1973; Roth et al. 1973, 1976; Elchisak et al. 1976; Korf et al. 1976; Westerink and Korf 1976). These increases in HVA concentrations are proportional to the duration and intensity of the electrical stimulation of DA neurons (Korf et al. 1976). Decreasing the impulse flow in DA pathways decreases DA metabolite concentrations (Bunney et al. 1973; Roth et al. 1973, 1976; Elchisak et al. 1976).

In lower animals, such as rats and cats, HVA concentrations in CSF or plasma have been shown to parallel the changes in brain tissue HVA concentrations (Guldberg 1969; Portig and Vogt 1969; Papeshi et al. 1971; Bacopoulos et al. 1979a; Kendler et al. 1981, 1982b). Similarly, destruction of brain DA neurons is associated with decreased urinary excretion of HVA (Hoeldtke et al. 1974). These data suggest that, in lower animals, HVA in CSF, plasma, or urine originates partly from the brain DA neurons and provides support for the notion that HVA concentrations in CSF, plasma, or urine can reflect the brain production rates of HVA.

In monkeys, parallel increases in plasma or CSF HVA concentrations occur in response to haloperidol, which is known to increase the firing rate and HVA production by brain DA neurons (Bacopoulos et al. 1978b). When most of the brain DA neurons were destroyed in monkeys by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
(MPTP), a significant decrease was demonstrated in CSF and plasma HVA concentrations (Kopin et al. 1988a) as well as urinary HVA excretion rates (Kopin et al. 1988b). MPTP is a selective neurotoxin that specifically destroys the brain DA neurons. These data indicate that a portion of HVA in CSF, plasma, and urine is contributed by the brain DA neurons in monkeys and that brain DA neuronal loss is reflected in a decrease in the HVA measurements in body fluids.

Similarly, in humans a limited loss of nigrostriatal DA neurons by accidental exposure to MPTP has been shown to lower CSF HVA concentrations (Burns et al. 1985). In addition, increasing the brain DA neuronal activity in humans by acute administration of neuroleptics increases HVA concentrations in CSF (Bowers 1973), plasma (Harris et al. 1984; Davidson et al. 1987a; Davila et al. 1987), and urine (Contreras et al. 1987). Elevations in plasma and urinary HVA measurements after administration of neuroleptics were also shown to originate, at least in part, from the brain and not from the periphery, since increases in the HVA measurements in plasma (Davidson et al. 1987a) and urine (Contreras et al. 1987) persisted even after debrisoquine suppressed the peripheral formation of HVA. Debrisoquine is known to suppress peripheral formation of HVA while leaving the brain production of HVA intact (Davidson et al. 1987c; Maas et al. 1988). These results support the view that a portion of HVA in CSF, plasma, and urine originates from the brain DA neurons, and an increase in brain DA neuronal activity or a loss of brain DA neurons can be reflected in an increased or decreased measurement of HVA in CSF, plasma, and urine.

Sources of HVA

HVA can be formed wherever its precursors are available and the necessary enzyme systems are present and active. Factors influencing the activity level of the involved enzyme systems and the availability of precursors dictate the overall amounts of HVA generated. For practical purposes, three major mechanisms—DA, NE neurons, and diet—contribute most of the HVA to its total body pool. DA and NE neurons are the main physiological sites containing the active enzyme systems that are needed for the formation of HVA and concentrated amounts of its precursors, whereas diet contributes to the total body pool of HVA by supplying excess precursors.

DA Neurons. The majority of DA neurons in the body are located in the brain. Although central dopamine neuronal activity is often referred to as if it were a single entity, there are several distinct groups of brain DA neurons that project to many areas of the brain. Even within well-identified groups of DA neurons, considerable heterogeneity appears to exist (Roth et al. 1987). Anatomical and physiological aspects of various groups of central DA neurons have been reviewed (Cooper et al. 1986; Roth et al. 1987; Falkovits and Brownstein 1989). The various groups of central DA neurons are only briefly outlined here.

The largest cluster of cell bodies of DA neurons is found in the substantia nigra and ventral tegmental regions of the midbrain. Three major projections of these DA neurons innervate the structures in striatum, limbic system, and cerebral cortex: the nigrostriatal, mesolimbic, and mesocortical DA neuronal systems, respectively. Other smaller collections of DA neurons are also present in the brain and are named according to their location and the destination of their projections; for example, the tuberoinfundibular (from hypothalamus to median eminence and pituitary gland), incertohypothalamic (linking the posterior hypothalamus to the anterior hypothalamus and septal nuclei), and medullary periventricular DA systems (small DA collections in the lower brainstem). In addition, small DA interneurons are also found in the retina and olfactory bulb. Since almost all the DA neurons are present in the brain, this source of HVA is often loosely referred to as the central HVA component. Strictly speaking, however, HVA produced in the central nervous system (CNS) originates not only from DA neurons but also from central NE neurons (discussed under “NE Neurons”).

A small number of peripheral dopaminergic nerves are now known to exist in human kidney, liver, and probably other tissues as well (Bell 1984, 1988; Bell et al. 1989; Bell and Mann 1990). Peripheral DA receptors are also identified in the kidney, the cardiovascular system, and the gastrointestinal tract (Berkowitz 1983; Struyker-Boudier 1986; Galvin and Szabo 1990). In some sympathetic ganglia, small nonneuronal, DA-containing cells also exist and may contribute significantly to the ganglionic levels of DA (Eranko 1976; Nef et al. 1983). However, the DA content of peripheral tissues is very small and constitutes approximately 1 to 5 percent of the total catecholamine pool in animals as well as humans (Bell 1988). Blocking the peripheral DA receptors with an agent that does not cross the blood-brain barrier does not significantly increase the plasma levels of HVA in humans (Konicki et al. 1991).
peripheral dopaminergic nerves might contribute small quantities of HVA to its body pool, especially in response to stimuli that preferentially stimulate these peripheral dopaminergic nerves (Bell 1988), but its quantity is unlikely to be substantial. These dopaminergic nerves are part of the peripheral sympathetic system that is predominantly noradrenergic. For all practical purposes, this peripheral source of HVA can be included in the sympathetic NE neuronal source discussed under “NE Neurons.”

Diet. Diet can supply excess DOPA, DA, and other precursors of HVA that are partly metabolized to HVA after ingestion. Diets consisting of foods high in monoamine content (e.g., cheese, orange juice, bananas, tomatoes) can substantially increase plasma (Kendler et al. 1983; Davidson et al. 1987b) as well as urinary HVA measurements (Johnston et al. 1983). Diets excluding foods that are known to have a high monoamine content (low-monoamine diet) do not generally increase plasma HVA concentrations. However, there appears to be some interindividual variability in the plasma HVA response to a low-monoamine diet; about one-third of the subjects show a modest increase in plasma HVA after ingesting a low-monoamine diet (Kendler et al. 1983; Davidson et al. 1987b). Pure carbohydrate foods (e.g., Polycose [Ross Laboratories]), which are devoid of monoamine precursors, do not affect plasma HVA concentrations. The effect of diet on plasma HVA lasts several hours, but extended overnight fasting of approximately 14 hours essentially eliminates the dietary effect on plasma HVA concentrations in humans as well as in monkeys (Davidson et al. 1987b; Elsworth et al. 1987).

NE Neurons. Reviews of NE neurons in the brain (Cooper et al. 1986; Palkovits and Brownstein 1989) as well as in the periphery (Weiner and Taylor 1985) are available. In the brain there are two main clusterings of NE neurons, locus coeruleus and lateral tegmental field, which innervate almost the entire brain. In the periphery, NE neurons make up the postganglionic link in the peripheral sympathetic system. The cell bodies of these NE neurons are located in the peripheral sympathetic ganglia. Projections of these NE neurons form an enormous network of NE nerve endings, which innervates virtually every tissue in the body.

NE neurons synthesize DA as a precursor to the formation of NE. Not all of the DA that is synthesized in both the central and the peripheral NE neurons is converted into NE; significant amounts are metabolized into DOPAC and then into HVA (Kopin 1985). Thus, the activity of NE neurons generates DA metabolites (e.g., DOPAC and HVA) in addition to the expected NE metabolites, such as 3-methoxy-4-hydroxyphenylglycol (MHPG) and vanillylmandelic acid (VMA). Also, a portion of DA formed in the peripheral NE neurons either is released as a co-neurotransmitter (Kvetnansky et al. 1979; Bell 1988) or leaks out into blood circulation and contributes to plasma concentrations of DA (Van Loon et al. 1979; Van Loon and Sole 1980; Van Loon 1983). Plasma DA is believed to be partly metabolized to HVA. The relative quantitative significance of these various mechanisms by which NE neurons contribute HVA to the total body pool of HVA is not clear.

In addition, a portion of DOPA, formed as an intermediary in sympathetic NE neurons, escapes into plasma (Goldstein et al. 1987). Plasma DOPA is believed to be converted into DA in peripheral tissues and may also contribute to the total body pool of HVA. However, the proportions of HVA formed by this route are currently not known.

The proportions of DA not converted into NE, in NE neurons, appear to vary. In rat tissues rich in the NE neuronal cell bodies (e.g., superior cervical ganglion and locus coeruleus), almost 50 percent of DA formed is not converted into NE (Anden et al. 1985). In tissues rich in the NE nerve endings, there is more efficient use of DA. In view of the large number of sympathetic ganglia in the human body and assuming their metabolism is similar to that in rats (Anden et al. 1985), the sympathetic NE neurons may constitute a very large source of HVA.

The data suggesting that noradrenergic neurons generate significant amounts of HVA come from both animal and human studies.

6-Hydroxydopamine is a selective noradrenergic as well as a dopaminergic neurotoxin. The main effect observed after it is administered systemically is the degeneration of peripheral noradrenergic nerve endings, as there are few DA neurons present in the periphery. Since this agent does not cross the blood-brain barrier, the brain remains unaffected by the systemic administration. In rats, after partial destruction of the noradrenergic neurons of the peripheral sympathetic system by 6-hydroxydopamine, there were significant reductions in plasma and urinary HVA measurements (Hoeldtke et al. 1974; Bacopoulous et al. 1979a).

The NE neuronal origin of HVA and other DA metabolites in rat tissues rich in NE neurons has been suggested by several investigators who used a variety of different tech-
niques (Lackovic et al. 1982; Anden and Grabowska-Anden 1983; Scatton et al. 1984; Anden et al. 1985; Curet et al. 1985; Dalmaz et al. 1985; Favre et al. 1986; Scheinin 1986). In rats, significant concentrations of HVA are found in tissues rich in NE neuronal cell bodies (e.g., locus coeruleus and sympathetic ganglia) as well as peripheral tissues that are rich in sympathetic nerve endings such as the carotid body, the urogenital tract, and the heart (Lackovic et al. 1982; Anden et al. 1985; Favre et al. 1986).

Pure autonomic failure (PAF) is a disease in which there is severe degeneration of peripheral sympathetic neurons with no known abnormalities in the brain DA neuronal systems. Patients with PAF produce not only very low amounts of NE metabolites (MHPG and VMA), as expected, but also very low amounts of HVA versus normal controls (Kopin et al. 1988c).

Debrisoquine, a peripheral MAO inhibitor that does not cross the blood-brain barrier, is preferentially concentrated in NE neurons of the peripheral sympathetic system (Medina et al. 1969; Pettinger et al. 1969). Debrisoquine suppresses the intraneuronal MAO in NE neurons that is required for the formation of deaminated metabolites of DA (DOPAC and HVA) and of NE (MHPG and VMA). In humans, debrisoquine has been used to suppress the peripheral formation of HVA. However, debrisoquine suppresses the NE metabolites (e.g., MHPG) to a proportionately greater degree than DA metabolites (e.g., HVA) in humans as well as in monkeys (Swann et al. 1980; Kopin et al. 1988a, 1988b; Maas et al. 1988; Amin et al., unpublished observation by authors). A linear positive correlation between the suppression of formation of MHPG and HVA by debrisoquine has been demonstrated (Kopin et al. 1988a, 1988b). Taken together with the fact that few DA neurons are present in the periphery, these data strongly suggest that the peripheral suppression of the formation of HVA by debrisoquine is in part secondary to its suppression of MAO activity in peripheral NE neurons.

In summary, even though HVA is generally considered an end product of DA neuronal metabolism, most of the total body pool of HVA is generated not by DA neurons but by NE neuronal systems. It appears that sympathetic NE neurons constitute the largest single source of HVA in the body. It has been estimated that the NE neuronal production of HVA constitutes approximately 75 percent of the total body production of the HVA in humans and nonhuman primates (Kopin et al. 1988a, 1988b; see section, “Contributions of Central DA Neurons to the Peripheral Pool of HVA”).

Other Sources of HVA. DA is synthesized in adrenal medulla as a precursor to NE and EPI. Adrenal medulla behaves in many ways as a sympathetic ganglion. In rats, very little HVA is found in adrenal medullary tissues, although some DOPAC has been demonstrated (Cottet-Emard and Peyrin 1982; Dalmaz et al. 1985; Favre et al. 1986). These data suggest a more efficient use of DA in adrenal medullary tissues toward the synthesis of NE and EPI as compared to sympathetic ganglia. However, adrenal medulla is believed to make significant contributions to the plasma DA levels during physical activity and the initial phases of prolonged stress (Van Loon et al. 1979; Van Loon and Sole 1980; Van Loon 1983). Therefore, it is possible that adrenal medulla contributes to the total body pool of HVA by releasing DA into plasma. However, the relative proportion of HVA formed via this route is currently unknown.

Brain capillary endothelium contains enzymes needed to synthesize and metabolize DA. These enzyme systems are part of the protective enzymatic blood-brain barrier (Bertler et al. 1963, 1966; Owman and Rosegren 1967; Pletscher and Bartholini 1970). Brain capillaries have been shown to contribute HVA, and a portion of HVA in CSF probably originates from the DA metabolism in the brain capillary walls (Goodwin et al. 1970; Tissot 1970; Bartholini et al. 1971). However, the proportions of HVA contributed by brain capillaries are not known.

Under pathological circumstances certain unusual sites may become a significant source of DA and HVA. For example, neuroblastoma and carcinoid tumors, which are secretory tumors of neural crest origin, have been shown to contribute large quantities of DA, HVA, and other monoamine metabolites such as VMA to their respective total body pools (Bohuon 1968; Schweisguth 1968; Feldman 1985).

Movement of HVA From Tissues Into Blood

HVA is an acidic compound and does not easily cross certain biological barriers, that is, from brain and CSF into blood and from blood into urine. A major determinant in the movement of HVA across these biological barriers is an organic anion transport system (Despopoulos and Weissbach 1957; Despopoulos 1959; Neff et al. 1967; Ebert et al. 1980). This organic anion transport system is present at various sites in the body.
Aizenstein and Korf (1978), studying percent of 5-HIAA (and by inference CSF (Meek and Neff 1973). Later, HVA formed in the brain entered in rats suggested that less than 10 percent of HVA (Neff et al. 1967; Meek and Neff 1973; Westerink and Kikkert 1986) and in the CSF (Olsson and Roos 1968; Post et al. 1975; Heninger et al. 1979; Berger et al. 1980) and plasma of humans (Kopin 1978; Heninger et al. 1979).

Movement of HVA From Brain Into CSF and/or Blood. Data from animals suggest that most of the HVA that is formed in the brain enters directly into the blood circulation without ever appearing in the CSF (Neff et al. 1967; Meek and Neff 1973; Aizenstein and Korf 1978). Earlier studies investigating 5-HIAA in rats suggested that less than 10 percent of 5-HIAA (and by inference HVA) formed in the brain entered CSF (Meek and Neff 1973). Later, Aizenstein and Korf (1978), studying HVA in rats, found that a maximum of 3.5 percent of the HVA formed in the brain entered CSF. In humans, the proportion of brain HVA that enters CSF is not known but is believed, by inference, to be very small, probably less than 3.5 percent. Some species differences are possible, however. In humans, an interindividual variation in this proportion is expected, and the magnitude of the proportion of HVA that enters CSF may be an important factor in determining CSF HVA concentrations.

A major mechanism responsible for the direct HVA egress from the brain tissues into blood appears to be the probenecid-sensitive organic anion transport system. This is evidenced in most animals by large accumulations of HVA and other acidic metabolites in the brain after probenecid treatment (Werdinian 1966; Andersson et al. 1973; Meek and Neff 1973; Westerink and Kikkert 1986). Other processes, such as diffusion along the gradient of HVA concentrations, which are much higher in the brain than in blood, may also play some role in the direct transfer of HVA from the brain into blood circulation (Wolffson et al. 1974; Kessler et al. 1976).

Many commonly used drugs in addition to probenecid have been shown in rats to influence the efflux of HVA from the brain into blood circulation. Haloperidol, chloral hydrate, and chlorpromazine strongly inhibited the egress of HVA from the brain into blood, while amphetamine stimulated this transport (Moleman et al. 1978; Westerink et al. 1984; Westerink and Kikkert 1986). Such effects of these drugs have also been shown to affect CSF HVA measurements in rats (Moore 1986). The nonspecific actions of many commonly used drugs on the organic anion transport system can thus be responsible for the observed increases in CSF HVA concentrations. However, the specific actions of certain drugs, such as probenecid, which is a serotonin metabolite, hippocapic acid, and urate, as well as xenobiotic compounds such as probenecid, para-aminohippuric acid (PAH), penicillin, salicylate, and phenolsulfophthalein dyes (Spector and Lorenzo 1974; Moller and Sheikh 1983). Understanding the movement of HVA and the factors that can affect this movement across certain barriers is important, since interfering with this process can significantly affect the HVA measurements in body fluids. For example, the drug probenecid, which is a prototype inhibitor of the organic anion transport system, has been demonstrated to cause the accumulation of HVA as well as other acidic monoamine metabolites in the brain and CSF of animals (Guldberg et al. 1966; Werdinian 1966; Neff et al. 1967; Bacopoulos et al. 1978a; Westerink and Kikkert 1986) and in the CSF (Olsson and Roos 1968; Post et al. 1975; Heninger et al. 1979; Berger et al. 1980) and plasma of humans (Kopin 1978; Heninger et al. 1979).

In animals, pharmacologically induced increases in the striatal HVA concentrations, either by increasing production of HVA (Guldberg and Yates 1968; Chase et al. 1970; Chase 1973; Bacopoulos et al. 1978a, 1980) or by inhibiting its egress with probenecid (Guldberg et al. 1966; Bacopoulos et al. 1978a; Ebert et al. 1980; Kendler 1982b), do increase the ventricular CSF HVA concentrations. This finding, together with the evidence that much of the CSF HVA is derived from the striatum, suggests a parallel between the HVA concentrations in the striatum and the CSF. In dogs, the striatal HVA concentrations correlated with that of the lateral ventricular CSF (Guldberg 1969), but in primates no correlation was found between concentrations in the CSF (and possibly other body fluids) and should be considered when interpreting data.

The brain HVA that enters CSF appears to originate mainly from the striatum adjacent to the lateral ventricles and contains the highest concentrations of HVA in the brain (Guldberg et al. 1966; Guldberg 1969; Papeschi et al. 1971; Sourkes 1973; Garelis et al. 1974; Curzon 1975; Bacopoulos et al. 1978b, 1980; Chase 1980; Wood 1980b). Consequently, the lateral ventricles have the highest concentrations of HVA in the CSF (Gordon et al. 1975; Ebert et al. 1980; Kopin 1985). In an unusual case of bilateral foramina of Monro occlusion, resulting in the isolation of lateral ventricles from the rest of CSF circulation, spinal CSF HVA concentrations were markedly reduced but nonetheless present (Garelis and Sourkes 1973). It is likely that small amounts of HVA may also enter CSF during its circulation through the third or fourth ventricle or both.

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striatal HVA and the cisternal CSF (Elsworth et al. 1987). However, dogs appear to be different from most other animals studied in that the probenecid-sensitive organic acid transport system is not significantly involved in the direct egress of HVA from the striatum (Guldberg 1969; Andersson et al. 1973) but appears to be involved in removing HVA from CSF (Andersson et al. 1973). It is likely that striatal and ventricular CSF HVA concentrations correlated with each other in dogs because most of the HVA is removed from the striatum via CSF and not via blood circulation. In most other species, including primates, most of the brain HVA is believed to exit directly into blood circulation and only a superficial portion of the striatum that lies next to the lateral ventricular wall probably contributes HVA to CSF. Thus, it is possible that in most species, including primates, CSF HVA concentrations would correlate only with a portion of the striatum that lies next to the lateral ventricular walls and not with the deeper regions of the striatum.

Since HVA that enters CSF circulates with it, it is important to consider the circulation of CSF in interpreting HVA data from various CSF regions. CSF circulation has been reviewed elsewhere (Wood 1980a; Milhorat and Hammock 1983), so only the salient features of CSF circulation are outlined here. CSF is mainly formed by the choroid plexuses of the ventricles. The CSF formed in the lateral ventricles passes through the third and fourth ventricles and then exits into the cisternal subarachnoid space around the brainstem. From there, a portion of CSF descends into the spinal canal and the rest ascends through the tentorial opening into the cortical subarachnoid space, where most of the CSF is absorbed into the blood circulation through venous sinuses. The CSF that circulates through the spinal canal descends mainly posterior to the spinal cord and then ascends anterior to the spinal cord. It flows via the anterior subarachnoid space of the brainstem into the cortical subarachnoid space (Wood 1980a; Milhorat and Hammock 1983).

HVA is absorbed from the CSF as it circulates so that its concentrations decrease along the path of the CSF circulation. This absorption results in a steep gradient of HVA concentration in the CSF from lateral ventricles to cisternal subarachnoid space and across the spinal canal in animals (Guldberg et al. 1966; Gordon et al. 1975; Ebert et al. 1980) as well as in humans (Siever et al. 1975; Sjostrom et al. 1975; Wode-Helgødt and Sedvall 1978; Nordin et al. 1982; Stanley et al. 1985; Kruesi et al. 1988). This concentration gradient is so steep that the spinal concentrations of HVA in primates have been estimated to be up to one-fiftieth of that found in lateral ventricles (Gordon et al. 1975; Ebert et al. 1980).

A major portion of HVA that enters CSF has been shown in several animal species to be removed by the probenecid-sensitive organic anion transport system (Pappenheimer et al. 1961; Ashcroft et al. 1968; Wolfson et al. 1974; Aizenstein and Korf 1978). This system appears to be located in the choroid plexus of the fourth ventricle (Pappenheimer et al. 1961; Guldberg et al. 1966; Ashcroft et al. 1968), the cortical subarachnoid space (Wolfson et al. 1974), and the spinal subarachnoid space (Van Der Poel et al. 1977; Aizenstein and Korf 1978). Passive diffusion and bulk flow of CSF also play a role but are responsible for quantitatively less significant movement of HVA from CSF into blood (Pappenheimer et al. 1961; Andersson et al. 1973; Wolfson et al. 1974).

The relative importance of various sites (i.e., ventricles and spinal or cortical subarachnoid spaces) involved in the removal of HVA and other acidic metabolites from the CSF have been studied. Wolfson and colleagues (1974) investigated the clearance sites of 5-HIAA (and, by inference, HVA) from the CSF by using perfusion studies in cats. It was found that clearances of 5-HIAA were lowest during the ventriculo-cisternal perfusions. The clearances were 10 times greater during the cortical subarachnoid perfusions. The spinal subarachnoid clearances were less than half of the cortical perfusions. These results suggest that most of the HVA in CSF is absorbed via the cortical subarachnoid space while the spinal subarachnoid space provides a quantitatively less important site and the ventricular clearance is least significant. This role of the cortical subarachnoid space is not unique to acidic metabolites, for other substances are also absorbed mainly into blood circulation via the cortical subarachnoid space (Lajtha and Toth 1961, 1962; Levin et al. 1966; Lorenzo and Snodgrass 1972). The large surface area and the rich vasculature on the surface of cortical hemispheres are the probable reasons why the cortical subarachnoid space plays a major role in the removal of HVA and other substances from the CSF.

It has been suggested that the acidic metabolites may also enter the cortical and spinal parenchyma from their respective subarachnoid spaces before being transported into the blood circulation (Wolfson et al. 1974; Kessler et al. 1976). This possibility is not surprising, since subarachnoid and brain extracellular
These data have led to speculation (Berger et al. 1988; Pickar et al. 1987) that cisternal-ventricular CSF HVA concentrations may directly reflect the mesocortical (particularly the DFC) DA neuronal activity as well (Pickar 1988; Weinberger et al. 1988; Pickar et al. 1990). The assumption implicit in these interpretations is that HVA can travel from the DFC to cisternal-spinal CSF regions. It is difficult to explain this assumption with the current understanding of the physiology of HVA in CSF. There are only two possible ways in which HVA from the DFC could travel to the cisternal-spinal CSF regions.

First, a portion of HVA formed in the DFC might enter the cortical subarachnoid space and travel down through the tentorial opening into the cisternal and spinal CSF regions. For this event to happen, HVA must move against the direction of CSF flow, which appears unlikely. Another evidence against any such movement is that the orbital frontal cortical (OFC) HVA concentrations did not significantly correlate with that of the cisternal CSF even though the OFC HVA concentrations are much higher than that of the DFC (Elsworth et al. 1987) and the OFC is closer to the cisternal CSF region than the DFC.

Second, HVA from the cortical regions may diffuse through the brain tissue into the lateral ventricles and then circulate with CSF to the cisternal and spinal regions. This possibility also appears unlikely, because the cortex is far apart from the lateral ventricular wall and are unlikely to represent the entire brain's DA metabolism.

Dorsal frontal cortical (DFC) HVA concentrations have been observed to correlate with the cisternal (Elsworth et al. 1987) and the spinal (Stanley et al. 1985) CSF HVA concentrations. These data have led to speculation that the cortex may contribute a significant amount of HVA to CSF (Elsworth et al. 1987) and that cisternal-spinal CSF HVA concentrations may directly reflect the mesocortical (particularly the DFC) DA neuronal activity as well (Pickar 1988; Weinberger et al. 1988; Pickar et al. 1990). The assumption implicit in these interpretations is that HVA can travel from the DFC into the lateral ventricles, because these structures are far apart and the concentration gradient is probably against any such movement.

On the contrary, the observed correlations between the cisternal CSF HVA concentrations and DFC (Elsworth et al. 1987) may be because the cisternal CSF ascends to the cortical regions, where HVA in CSF can freely mix and equilibrate with that of the DFC. Similarly, the observed correlations between the spinal CSF and the DFC HVA concentrations (Stanley et al. 1985) may be because it is the cisternal CSF that travels partly to the spinal and partly to the cortical regions. These two portions of cisternal CSF would be similar in their HVA concentrations and may explain the correlation observed by Stanley and colleagues (1985). The failure of significant correlations between the HVA concentrations in the OFC and cisternal CSF may be due to the OFC having much higher HVA concentrations (Elsworth et al. 1987) than the DFC and may be less reflective of CSF HVA concentrations than the DFC. Thus, it is possible that the observed correlations of HVA between DFC and CSF merely reflect the circulatory pattern of CSF HVA and the continuity between CSF and cortical regions (and not that significant contributions of CSF HVA come from cortex).

In summary, it is unclear whether lumbar and cisternal CSF HVA concentrations can directly reflect the cortical DA neuronal activity. It is possible that the observed associations between the clinical findings related to the cortical areas and CSF HVA concentrations (Pickar 1988; Weinberger et al. 1988; Pickar et al. 1990) may be due to an indirect and a more complex relationship. Further
work is needed to clarify the nature of observed associations between CSF HVA concentrations and the clinical findings related to certain cortical areas.

Movement of HVA From Peripheral Tissues Into Blood. The probenecid-sensitive organic anion transport system is also involved in the movement of HVA from most peripheral sympathetic ganglia into the blood circulation. This point is suggested by the accumulation of HVA in the sympathetic ganglia in rats after probenecid treatment (Dalmaz et al. 1985). However, the probenecid-sensitive transport system appears to be responsible for only up to one-third of the HVA egress from peripheral sympathetic ganglia; most of the HVA enters blood circulation either by diffusion or by bulk movement of the tissue fluid (Dalmaz et al. 1985).

Kinetics of HVA

The movement of HVA in the body is represented in figure 2. After entering blood circulation, HVA equilibrates with the plasma volume that serves as a primary distribution compartment as well as with the larger extravascular fluid volume that serves as a secondary distribution compartment.

The kinetics of HVA in the body were studied by injecting isotope-labeled HVA intravenously in monkeys (Elchisak et al. 1978, 1979) as well as in humans (Anggard et al. 1975; Elchisak et al. 1982). The mean plasma half-life of HVA in human volunteers appears to be about 1 hour (Anggard et al. 1975; Elchisak et al. 1982). The average volume of distribution of HVA in humans appears to be about 35 liters or approximately 0.5 L/kg of body weight (Anggard et al. 1975; Elchisak et al. 1982). This volume of distribution calculated from these kinetic studies far exceeds the expected extracellular fluid volume in normal individuals and suggests the HVA might be significantly distributed in the intracellular volume in addition to the extracellular volume. Also, the possibilities of sequestration of HVA somewhere in the body and of complex kinetics cannot be ruled out. The kinetics of HVA in the body need to be studied further.

Excretion

Renal Excretion. Renal excretion of HVA has been studied by injecting isotope-labeled HVA intravenously and subsequently collecting urine samples in monkeys (Elchisak et al. 1978, 1979; Miller et al. 1987) and in humans (Anggard et al. 1975). Earlier studies using deuterated HVA in humans (Anggard et al. 1975) and in nonhuman primates (Elchisak et al. 1979) showed that only about half of the injected, labeled HVA was recovered in the urine, suggesting the presence of one or more alternate mechanisms of HVA elimination in primates. Miller and colleagues (1987) argued that this phenomenon might have resulted from altered kinetics due to overexpansion of total body pool of HVA by the large dose of deuterated HVA used in earlier studies. They injected very small amounts of tritiated HVA in monkeys and were able to recover almost 95 percent of the injected, labeled HVA in urine within 4 hours. However, the results of these studies are difficult to compare because the last study used tritium-labeled HVA, whereas deuterium labeling was used in the earlier studies. Tritium-labeled HVA may have different molecular polarity than deuterated HVA and thus could have behaved differently.

CSF = cerebrospinal fluid; HVA = homovanillic acid.
in the body, therefore accounting for a different outcome. Plasma kinetics of tritiated HVA in monkeys during the study by Miller and colleagues (1987) are not available for comparison with the earlier studies to clarify this issue. It is possible that most of the HVA in humans and nonhuman primates is excreted by the kidneys into urine.

The urinary excretion of HVA involves glomerular filtration, active tubular secretion (by the organic anion transport system), and possible reabsorption and is similar to the large number of other organic acidic compounds (Irish and Grantham 1981; Moller and Sheikh 1983; Weiner and Mudge 1985; Grantham and Chonko 1986; Potter et al. 1989). In primates, renal tubular secretion of HVA by an organic anion transport system appears to be the major mechanism of HVA excretion from the body, since large increases in plasma HVA concentrations are observed after probenecid treatment (Bacopoulos et al. 1978a; Kopin 1978). Probenecid is a competitive inhibitor of this organic anion transport system, thereby causing the accumulation of HVA in plasma. This organic anion transport system is located in the proximal renal tubules and is shared by a large number of other organic acidic compounds (e.g., 5-HIAA, urate, PAH, probenecid, penicillin, salicylate, indomethacin, diodrast), all of which compete with one another for the same transport sites for secretion (Irish and Grantham 1981; Moller and Sheikh 1983; Grantham and Chonko 1986). Large amounts of any one of these compounds can interfere with the excretion of other compounds sharing the same organic anion transport system; for example, the excretion of probenecid can be inhibited by PAH and vice versa (Weiner et al. 1960).

Indomethacin, which also shares this transport system, has recently been observed to significantly increase plasma concentrations of HVA (Kahn et al. 1991). This finding is consistent with the competitive inhibition of renal tubular secretion of HVA by indomethacin, analogous to that of probenecid.

Many commonly used psychiatric drugs (e.g., haloperidol, chloral hydrate, chlorpromazine) can affect the probenecid-sensitive organic anion transport system in the brain. It is not known whether these drugs can also inhibit renal tubular secretion of HVA and, therefore, interfere with plasma and urinary HVA measurements. A large number of pharmacologic agents can potentially affect this nonspecific organic anion transport system and might affect HVA measurements in plasma and urine (Moller and Sheikh 1983). Possible renal effects of commonly used psychiatric drugs need to be studied. Pharmacologically induced changes in plasma HVA concentrations must be interpreted cautiously.

Common endogenous metabolites such as lactate and ketoacids can also interfere with the tubular secretion of organic acids (Irish and Grantham 1981; Moller and Sheikh 1983). Sodium lactate infusion significantly decreased urinary excretion of HVA in normal controls as well as in patients with panic disorder (Clow et al. 1988). Although not discussed by Clow and colleagues, these data are consistent with the possibility that lactate infusion raised plasma lactate to levels that interfered with renal tubular secretion of HVA. The effect of common endogenous metabolites such as ketoacids and free fatty acids on HVA excretion needs to be studied.

Many organic anions (e.g., probenecid and salicylate) are re-absorbed after they are secreted in the proximal tubules. It is unclear at this point whether HVA is re-absorbed after tubular secretion. One piece of evidence suggesting that reabsorption of HVA might occur is that urinary excretion of HVA was reported to increase with high rates of urine formation in humans (Anggard et al. 1975). This pattern is usually seen with organic anions that are passively reabsorbed after they are secreted (e.g., probenecid and salicylate [Irish and Grantham 1981; Grantham and Chonko 1986]). Therefore, the rate of urine flow might be an important determinant of HVA excretion and, hence, its measurement in plasma and urine. The effect of urine flow on HVA excretion needs to be investigated.

It is well known that urinary excretion of organic anions can be substantially enhanced by alkalinization of urine (Irish and Grantham 1981). For example, the urinary excretion of salicylate can be increased four to six times by increasing urinary pH from 6.4 to 8.0 (Flower et al. 1985). Similarly, urinary excretion of probenecid can also be significantly increased by alkalinizing urine (Weiner et al. 1960). Thus, urinary excretion of HVA could be affected by changes in the urinary pH. There is a physiological variation in the urinary pH depending on the type of diet, the metabolic state, and the intake of acidic or alkaline substances of the individual. The effect of changing urinary pH on the excretion of HVA and its plasma concentrations needs to be investigated.

In summary, since HVA is very rapidly cleared from plasma (plasma half-life is 1 hour), the rate of renal excretion is an important determinant of plasma HVA concentrations. Despite a large body of HVA data from animal and human studies, re-
nal excretion of HVA has been largely ignored. A large number of factors such as urinary pH, urine flow rate, pharmacologic agents, and endogenous metabolites can all potentially affect the urinary excretion of HVA and, therefore, its concentrations in plasma. Which of these factors are important and need to be controlled in studies using HVA in plasma or urine as a possible index of central DA neuronal activity must be investigated. The effect of these renal factors on plasma HVA concentrations might explain the commonly observed significant fluctuations in plasma HVA measurements.

Nonrenal Excretion. The organic anion transport system, which plays a major role in the urinary excretion of HVA, also exists in the liver (Sperber 1959; Woo and Hong 1963; Despopoulos 1966, 1971; Irish and Grantham 1981; Moller and Sheikh 1983; Grantham and Chonko 1986), where it secretes the same compounds into bile that are also secreted by the kidney, such as PAH (Despopoulos 1966), bromosulfalein (Woo and Hong 1963), indomethacin (Flower et al. 1985), and probenecid (Weiner and Mudge 1985). Whether significant amounts of HVA are also secreted via bile is currently unknown. Possible biliary secretion of HVA and its significance in determining plasma concentrations of HVA need to be investigated.

Characteristics of HVA Measurement in CSF, Plasma, and Urine

Three body fluids are available for HVA measurements in humans: CSF, plasma, and urine. The major characteristics of HVA measurements in each of these body fluids and their limitations are reviewed below.

CSF. The proximity and continuity of CSF to the brain make it a logical place to look for metabolite changes representing brain DA neuronal activity. The movement of HVA from the brain into and out of the CSF is discussed in “Movement of HVA From Tissues Into Blood.” Since HVA is very unevenly distributed in CSF, the location from which CSF is obtained greatly influences concentrations of HVA. It is important to note that in clinical practice CSF is obtained from the lumbar region of the spinal canal.

HVA measurement in CSF is advantageous because it originates from within the CNS and is not affected by substantial amounts of HVA produced in the peripheral tissues. Although HVA in CSF is contributed not only by the central DA neurons but also by the central NE neurons (discussed under “NE Neurons”), the total number of central DA neurons and their rates of tyrosine hydroxylase activity are much greater than that of central NE neurons (Cooper et al. 1986). Therefore, much of the HVA in CSF is likely to originate from the central DA neurons. Measurements of HVA in the CSF would thus appear less “contaminated” than in blood or urine.

CSF HVA measurement, however, has several serious shortcomings. The access to CSF is very limited, thereby restricting the amount of information that can be obtained from CSF HVA measurement. The limited access is, in part, the reason why several important aspects of CSF HVA physiology in humans are still unclear (see section, “Movement of HVA From Tissue Into Blood”) despite more than two decades of research. CSF HVA concentrations are dynamic, and striking fluctuations in the same person under controlled conditions have been reported (Hil-debrand et al. 1990). Diurnal variations in the ventricular CSF HVA concentrations have been demonstrated in primates (Perlow et al. 1977; Perlow and Lake 1980) but are difficult to study in humans. Such fluctuations in CSF HVA concentrations call for repeated CSF measurements in clinical research that cannot be easily done. The need for repeated measurements is further discussed under section, “Limitations of Measuring HVA in Body Fluids.” As discussed earlier (see “Movement of HVA From Tissue Into Blood”), most of the HVA formed in the brain bypasses CSF and enters the blood circulation directly. A variable proportion, possibly less than 3.5 percent of brain HVA, enters CSF, and much of that is believed to originate from the portion of the striatum that is adjacent to the lateral ventricular walls. Thus, CSF HVA is unlikely to represent the entire brain’s DA metabolism and at best reflects primarily the striatal DA metabolism. It is unclear whether CSF HVA concentrations can directly reflect the cortical DA metabolism.

Many complex confounding factors may play significant roles in determining the lumbar CSF HVA concentrations. The proportion of HVA that leaves the brain via CSF is likely to be a major determinant of CSF HVA concentrations, irrespective of brain DA neuronal activity, and may be a major source of variance. The characteristics of HVA egress from CSF during its circulation may also be important. In one extreme case with deficient removal of HVA from CSF, very high CSF HVA concentrations were observed (Bowers 1969). Mutual competition among various anions sharing the same organic anion transport system may also play a role. Although CSF HVA originates from within the CNS
and probably much of CSF HVA is contributed by central DA neurons, other sources still probably contribute significant but variable amounts of HVA in CSF. For example, brain NE neurons, brain capillary endothelial metabolism (see “Other Sources of HVA”), and the extensive sympathetic neuronal network that accompanies brain vasculature (Edvinsson et al. 1983; Nathanson 1983) are all likely sources of HVA in CSF. The relative contributions and factors influencing these “contaminating” sources of HVA in CSF are currently unknown. All confounding factors that can affect CSF HVA measurements may, in part, be the reason for the very wide interindividual variation found even in normal volunteers under strict research conditions (Ballenger et al. 1982). This may make consistent group differences difficult to elicit (Hildebrand et al. 1990).

The concentration gradient of HVA in CSF (see “Movement of HVA From Brain Into CSF and Blood”) along the spinal canal is so steep that CSF HVA concentrations obtained from L5–S1 lumbar space were shown to be 29 percent lower than that of CSF obtained from L4–L5 lumbar space (Nordin et al. 1982). Thus, factors such as the length of the spinal canal and the height of the individual can play significant roles in determining the CSF HVA concentrations (Wode-Helgodt and Sedvall 1978). This concentration gradient results in higher HVA concentrations in the successive CSF fractions. To control for this confounding variable, similar fractions of CSF samples are analyzed. However, since patients may differ in the length and the width of the spinal canal and the volume of CSF contained, similar fractions may not be equidistant from the lateral ventricles. Because the decrease in HVA concentrations along the spinal canal can be so steep and variable, this gradient might still be a source of significant variance.

In summary, despite the proximity of CSF to the brain and the general perception of CSF as the central body fluid, many complex confounding factors can seriously limit the usefulness of the lumbar CSF HVA concentrations as possible indicators of central DA neuronal activity. Lumbar CSF HVA measurements probably reflect only major changes in DA neuronal activity from a limited region (primarily striatal) of the brain. Lumbar CSF HVA measurements are unlikely to represent the entire brain’s DA metabolism and the DA neuronal activity from all the central DA projections.

**Plasma.** Since HVA from most regions of the brain enters directly into blood and HVA that enters CSF is then absorbed into blood, plasma is a logical site to measure HVA as a possible index of central DA neuronal activity. The main factors that determine HVA concentrations in plasma are the rates at which HVA enters plasma (i.e., rates of HVA production in the body) and the rates at which it is cleared from blood circulation. In addition, complex factors such as the volume of plasma (the primary compartment of distribution), the size of the extra-vascular fluid volume (the secondary distribution compartment or compartments), and the rates of equilibrium between compartments can possibly influence plasma HVA concentrations as well.

Since repeated and frequent measurements can be easily obtained, plasma HVA measurements are well suited for studying the time course of rapid changes in HVA production rates in response to various stimuli.

The main disadvantage of measuring HVA in plasma is that plasma HVA is contributed not only by central DA neurons but also by other sources, notably NE neurons and diet. Although extended overnight fasting can essentially eliminate dietary HVA contributions, HVA contributions by NE neurons, mostly of the peripheral sympathetic system, remain a problem. In primates, only about 25 percent of plasma and urinary HVA originates from the central DA neurons. The rest, approximately 75 percent, comes from the peripheral sympathetic system. The drug debrisoquine has been used to suppress the peripheral contributions of HVA to plasma. However, the presence of significant residual formation of HVA in the periphery after debrisoquine treatment complicates and limits the utility of this approach. A more recent strategy offers hope and direction for further work to eliminate this most serious limitation in using plasma HVA concentrations as an index of central DA neuronal activity (see section, “Contributions of Central DA Neurons to the Peripheral Pool of HVA”). Until better methods are available, the measurement of a peripheral noradrenergic index (such as MHPG) simultaneously with plasma HVA measurement might help clarify whether any observed change in total plasma HVA is the result of its central dopaminergic component or peripheral noradrenergic component.

Plasma HVA concentrations are determined not only by rates of HVA entry into plasma (rates of production) but also by rates of HVA elimination. Since HVA is very rapidly eliminated from blood circulation, as evidenced by very low plasma concentrations and a half-life of approximately 1 hour, factors af-
fecting HVA elimination can significantly affect plasma HVA concentrations. Many factors such as competition among the concentrations of various endogenous organic anions sharing the same organic anion transport system (Moller and Sheikh 1983), renal variables (see section, "Excretion"), and a large number of drugs may all be important. The measurement of renal plasma clearance has been suggested to control for renal variables (Potter et al. 1989). However, this approach has the same limitations as that of measuring urinary HVA concentrations (see "Urine"). An alternate approach may be to investigate the utility of a suitable "control" endogenous organic anion in plasma that uses the same organic anion transport system for its excretion. Since the organic anion transport system appears to be the major eliminating mechanism for HVA and other organic anions, not only via kidney but from nonrenal routes as well (see section, "Excretion"), identification of a suitable anion may help control the elimination variables.

Significant fluctuations in plasma concentrations of HVA can occur in the same individual even within short time intervals (Riddle et al. 1987; Baker et al. 1988). It is possible that the variability observed in plasma is due, at least in part, to changes in the excretion of HVA and not to changes in HVA production rates.

Urine. Since most of the HVA is believed to be excreted in urine, the measurement of urinary HVA excretion rates may be useful in estimating production rates of HVA. Urinary excretion rates of HVA can be expressed per unit time (e.g., per 24 hours or 12 hours) by collecting urine samples for a specified time. Urinary excretion rates of HVA can also be more conveniently expressed per milligram of creatinine excreted in urine, since daily creatinine excretion remains fairly constant.

The main advantages of measuring urinary HVA excretion rates are that it is both noninvasive and painless. Urinary HVA excretion rates would equal HVA rates of formation in the body during a sufficient time period, assuming a state of equilibrium and urinary excretion as the primary route of elimination of HVA from the body. For example, urinary HVA excretion rates during 24 hours are likely to equal average daily production rates. Urinary HVA excretion during a sufficient time period is a pooled measurement and is less affected by the possibility of rapid fluctuations, as in the case of plasma HVA measurements. Because of a possible cumulative effect, urinary HVA excretion rates over several hours have been suggested to be more sensitive in detecting minor changes in HVA production rates than a single plasma HVA measurement (Contreras et al. 1988).

The main disadvantage of urinary HVA measurements is that the collection of adequate urinary samples is a lengthy process that requires reliable and cooperative subjects. This requirement makes urinary HVA measurement for psychotic patients inherently difficult. In these patients, ensuring adequate collection of urine samples can be difficult, frustrating, time-consuming, costly to monitor, and often unreliable. HVA in urine, like in plasma, comes not only from the central DA neurons but from all other sources as well. Thus, the utility of urinary HVA measurement is limited as an index of central DA neuronal activity, as in plasma HVA measurement. Additional routes of HVA elimination (e.g., via bile) might be significantly operative as well. The significance of these routes on the utility of urinary HVA measurement as an indicator of central DA neuronal activity needs to be studied.

Limitations of Measuring HVA in Body Fluids: Some Solutions. It is obvious from the preceding discussion that many factors unrelated to brain DA neuronal activity can strongly influence the concentration of HVA in each of the body fluids. Therefore, it is not surprising that all the preclinical and clinical studies are not entirely consistent. Despite the evidence that HVA in body fluids is in part derived from the brain DA neurons (see "HVA Measurement as Possible Indicator of Central DA Neuronal Activity") and parallels of HVA concentrations that have been demonstrated in animals and humans between brain and CSF (Guldberg et al. 1969; Bacopoulos et al. 1979a, 1980), brain and plasma (Roth and Bacopoulos 1979; Bacopoulos et al. 1980; Kendler et al. 1982a, 1982b; Kendler and Davis 1984), and CSF and plasma (Bacopoulos et al. 1978a, 1980; Heninger et al. 1979; Bacopoulos et al. 1979a, 1980; Kendler et al. 1982b; Degrell and Nagy 1990), a few contrary findings have also been reported. For example, HVA concentrations did not correlate among brain, CSF, and plasma in primates (Elsworth et al. 1987). In humans, HVA concentrations did not correlate between CSF and plasma (Maas et al. 1988). One group did not observe increases in plasma HVA concentrations after acute neuroleptic treatment (Pickar et al. 1986), but the same group did find such an increase in a later study (Konicki et al. 1991). In one study of a small number of human subjects with accidental exposure to MPTP, decreases of
HVA in CSF were found, but not in the urine (Burns et al. 1985).

In many of the above-mentioned animal studies, in which parallels were observed between brain and body fluid HVA concentrations, gross manipulations in the brain HVA production were used, although generally with a very small number of observations. In clinical studies using a sufficient number of observations, HVA measurements in body fluids can probably provide information about the major changes in the overall central DA neuronal production of HVA.

When HVA measurements are used in body fluids, repeated measurement designs within the same subjects may offer a better reflection of central DA neuronal activity than cross-sectional study designs. Cross-sectional clinical study designs, in which HVA concentrations in a body fluid at a certain time point are studied in relation to clinical parameters, may be relatively less powerful, because they may allow room for many confounding factors to cloud the results. Repeated measurement designs, on the other hand, use HVA concentrations measured at two or more time points and compare the changes in HVA concentrations with the changes in clinical parameters within the same subjects under otherwise controlled conditions. This design may be relatively more powerful, since it may hold constant many of the major confounding factors that may otherwise obscure the results. For example, in clinical studies with a repeated measurement design, even when CSF and plasma HVA concentrations did not correlate with each other cross-sectionally, the changes of HVA concentrations in CSF and plasma correlated with each other significantly (Sharma et al. 1988, 1989).

Since HVA concentrations in body fluids are dynamic, averaging two or more measurements might help control the effects of confounding fluctuations in each body fluid. Only very limited data on the reproducibility of HVA measurements in body fluids are currently available. Further efforts need to be directed at studying and possibly enhancing the reproducibility of body fluid HVA measurements.

As discussed earlier, there are several different DA projections in the brain, each of which perhaps performs different functions (see section, "DA Neurons"). The relative contributions of the major DA pathways to the total brain production of HVA are currently not known. Obviously, HVA measurements in body fluids do not point out which of the DA pathways are involved under given conditions. However, once clinical, biological, or neuropsychological correlates of some of the individual DA pathways are identified, useful information about specific central DA pathways may possibly be obtained by simultaneously measuring HVA and such correlates of specific DA pathways. Another, and perhaps a more immediate, prerequisite toward this goal is to improve the methods of measuring HVA in body fluids to reliably reflect the overall central DA activity with sufficient sensitivity. This improvement could be achieved by investigating and controlling the major confounding factors that can affect HVA measurements in body fluids.

Since different sets of factors can affect HVA measurements differently in each of the three pertinent body fluids, using more than one body fluid in a clinical study might provide more useful information. For example, if HVA is derived in CSF mainly from the nigrostriatal projections (see "Movement of HVA From Brain Into CSF and Blood") and in plasma from all the brain DA projections in addition to the peripheral contributions, similar clinical findings in plasma and CSF may suggest nigrostriatal involvement (assuming that CSF does not directly reflect the mesocortical DA activity). Findings in plasma (when peripheral artifacts can be ruled out) but not in CSF might suggest involvement of other major central DA projections, namely, mesocortical or mesolimbic or both.

Since HVA from most regions of the brain may enter directly into blood circulation without ever appearing in CSF and since a variable amount of HVA may also bypass urine, HVA measurements of plasma appear potentially most suitable for studying overall central DA neuronal activity. However, the biggest limitation in using plasma HVA measurements as possible indicators of central DA neuronal activity is that a substantial amount of plasma HVA originates from the periphery. The knowledge of how much of the HVA in plasma is contributed by central DA neurons and the strategies that can measure the central HVA component in plasma are of obvious importance. The following is a discussion of strategies used in primates to determine the central DA neuronal contributions of HVA in plasma.

**Contributions of Central DA Neurons to the Peripheral Pool of HVA**

In humans, estimations of central DA neuronal contributions to the total plasma HVA concentrations have been attempted by suppressing the peripheral formation of HVA by debrisoquine. That the maximum suppression of plasma HVA concen-
trations achieved by debrisoquine in human studies has been approximately 55 percent of baseline plasma HVA concentrations suggests that approximately 45 percent of plasma HVA originated from the brain DA neurons (Davidson et al. 1987c; Maas et al. 1988). However, this method appears to grossly overestimate the central DA neuronal contributions to the total plasma HVA concentrations for two reasons. First, the suppression of peripheral formation of HVA by debrisoquine is not complete. Even after maximum peripheral suppression of HVA formation by debrisoquine, residual amounts of HVA are still formed in the periphery (Hovevey-Sion et al. 1989). Second, there are significant amounts of HVA produced by central NE neurons. Since debrisoquine does not enter the brain, it does not suppress HVA formation by central NE neurons. The amount of HVA produced by central NE neurons is included in the residual plasma HVA concentrations obtained after debrisoquine treatment. Thus, the suggestion that approximately 45 percent of the total plasma HVA originates from the central DA neurons is probably a gross overestimation.

HVA production by the brain has also been calculated from the differences between jugular venous and arterial concentrations of HVA and the total cerebral blood flow in monkeys (Maas et al. 1979) as well as in humans (Maas et al. 1980). These data suggest that the average brain contributions of the total 24-hour urinary HVA excretion amounted to about 25 percent in monkeys and 33 percent in humans. However, the brain contributions of HVA to the total daily excretion of HVA by this method represent the amount of HVA produced not only by central DA neurons but by central NE neurons as well. Hence, the central DA neuronal contributions of HVA to the total daily excretion of HVA would be less than 33 percent in humans.

Since most of the peripheral HVA derives from sympathetic NE neurons, Kopin and colleagues (1988c) attempted to estimate the central DA neuronal productions of HVA from the relationship between the NE metabolites (MHPG and VMA) and HVA. Urinary excretion rates of total NE metabolites (MHPG + VMA) and HVA were measured in normal controls as well as in patients with varying degrees of NE neuronal degeneration but presumably intact brain DA neuronal systems. Patients with NE neuronal degeneration excreted smaller daily amounts of NE neuronal metabolites (MHPG, VMA) as expected, but they also excreted smaller daily amounts of HVA, probably because of smaller NE neuronal contributions of HVA. Sums of the total daily excretion rates of MHPG + VMA were plotted against the total daily HVA excretion rates, with MHPG + VMA values on the x axis and HVA values on the y axis. A highly significant positive linear correlation between the sum of NE metabolites (MHPG + VMA) and HVA was observed. This finding is consistent with the origin of a portion of HVA in common with MHPG and VMA from the peripheral NE neurons. A regression line of this positive linear correlation between MHPG + VMA and HVA was obtained. The regression line did not reach a zero x axis value, because even in patients with severe peripheral NE neuronal degeneration some residual NE neuronal metabolism was present. The regression line was then extrapolated to obtain a y intercept, which is the value of the y axis (HVA) at the zero value of the x axis (the hypothetical absence of all NE neuronal metabolism). The magnitude of the y intercept was found to be significantly greater than zero. Kopin and colleagues (1988c) argued that the magnitude of the y intercept was the component of HVA formed in the hypothetical absence of all NE metabolism and represented the amount of HVA produced by the central DA neurons. Calculated by this method, the amount of HVA produced by the central DA neurons represented about 25 percent of the total daily HVA excretion by normal humans.

Kopin and colleagues (1988a, 1988b) replicated these findings with slight modifications to calculate central DA neuronal contributions of HVA to the total plasma and urinary HVA measurements in individual monkeys. Debrisoquine was used to suppress the formation of HVA and MHPG produced by the peripheral NE neurons. Debrisoquine was administered to monkeys in gradually increasing doses, and HVA and MHPG were measured daily in their plasma and urine for 8 days. Thus, a gradual decrease in the rates of formation of MHPG and HVA by peripheral NE neurons was obtained while the central DA neuronal production of HVA was left intact.

The values of HVA and MHPG were plotted against each other, MHPG on the x axis and HVA on the y axis. A positive linear correlation between HVA and MHPG was observed, consistent with a common origin of a part of HVA with that of MHPG from peripheral NE neurons. The regression line of this correlation was obtained and extrapolated to obtain the y intercept (value of y at x = zero). The y intercept was found to be significantly above zero and represented the component of HVA that was independent of pe-
ripheral NE neuronal metabolism. The magnitude of y intercept that reflected the component of HVA produced mostly by the central DA neurons (which was not affected by the debrisoquine administration) amounted to about 25 percent of the baseline plasma and urinary HVA measurements. To confirm the central dopaminergic origin of HVA measured by this strategy, the central DA neurons were selectively destroyed with MPTP. It was demonstrated that the magnitude of the y intercept decreased by more than 80 percent after the treatment with MPTP, thus confirming the central DA neuronal origin of the HVA measured by this strategy. However, even this method may overestimate the central DA neuronal contributions to the total plasma or urinary HVA measurements, because it includes the contributions of HVA from DOPA leaked from the sympathetic NE neurons and metabolized in nonneuronal tissues to HVA (Hovevey-Sion et al. 1989). Nonetheless, this last strategy is potentially very useful, since similar paradigms can be used in humans (Kopin et al. 1988d).

By using the same strategy, plasma HVA and MHPG were measured in a group (n = 9) of drug-free schizophrenic patients during debrisoquine treatment (Amin et al. 1991). Plasma HVA correlated significantly with MHPG and the y intercept was significantly greater than zero. The magnitude of y intercept that represented the central HVA component comprised only 24 percent (3.08 ng/mL) of the mean baseline plasma HVA concentrations (13.02 ng/mL). These results support the evidence that, on average, only about one-quarter of the total plasma HVA comes from central DA neurons. Further work is needed to establish the usefulness of this strategy in clinical research. This strategy appears to offer hope and direction for further work to obtain better measures of central DA neuronal activity in plasma.

Conclusions

• Despite its severe limitations, the measurement of HVA in body fluids is perhaps the most direct method currently available in living humans to assess the changing activity of central DA neurons. HVA concentrations in body fluids probably reflect only major changes in the overall central DA turnover and, hence, central DA neuronal activity. Various factors unrelated to brain DA neuronal activity can confound the body fluid HVA measurements and may compromise the usefulness of those measurements in detecting smaller yet perhaps important changes in central DA neuronal activity. Further methodological work is needed to enhance the utility of this potentially valuable tool in neuropsychiatric research. Possible effects of relevant confounding factors must be considered in interpreting HVA data.

• In the use of body fluid HVA measurements as possible indicators of central DA neuronal activity, repeated measurement designs within the same subjects may offer a better reflection of central DA neuronal activity than the cross-sectional study designs. Cross-sectional study designs can be affected by many confounding factors that affect HVA measurements, while repeated measurement designs can hold constant many of these confounding factors that may otherwise cloud results.

• CSF HVA appears to originate from a restricted region of the brain, mainly from a portion of the striatum adjacent to the lateral ventricular walls. CSF HVA concentrations probably reflect only major changes in the striatal DA neuronal activity. It is unclear whether CSF HVA concentrations directly reflect cortical DA neuronal activity. The nature of the observed associations of CSF HVA with the clinical findings related to certain cortical areas needs further clarification, remains quite intriguing, and should stimulate further research.

• Since a significant portion of HVA in plasma and urine is derived from the brain, HVA measurements in these peripheral body fluids may be convenient and potentially useful indicators of central DA neuronal activity.

• Urinary HVA measurements are limited by the large peripheral HVA contributions and the possibility that a variable portion of HVA might be excreted via nonrenal routes. Urinary HVA measurements require prolonged urine collection, demand extended subject cooperation, and, therefore, may seem less suitable for psychotic patients.

• Since HVA from most regions of the brain may enter directly into blood circulation without ever appearing in CSF, and since a variable amount of HVA may also bypass urine, plasma may be the most useful body fluid for HVA measurement as an index of an overall central DA neuronal activity. However, large HVA contributions to plasma from NE neurons and possible renal confounding factors are major limitations.

• Plasma HVA concentrations are determined not only by the rates of HVA entry into plasma but also by its rates of elimination. Factors that influence HVA elimination and therefore its plasma concentrations have
not been adequately studied. Further methodological studies need to be directed at clarifying the factors involved in HVA excretion and the significance of those factors on plasma HVA concentrations. It is possible that fluctuations in plasma HVA concentrations might be due in part to changes in HVA excretion rates rather than to changes in the production rates.

- Even though HVA is generally considered to be a DA neuronal metabolite, much of the HVA in the body appears to be produced by the NE neurons and not by the DA neurons. Under fasting conditions, total HVA measurements in plasma or urine consist of two major components: dopaminergic HVA that is predominantly of central DA neuronal origin, and noradrenergic HVA that is mostly of peripheral NE neuronal origin. Therefore, it is important to measure the NE metabolites (such as MHPG) in addition to HVA to help clarify whether the observed changes in total HVA measurements were the result of changes in its central dopaminergic component or in its noradrenergic component.

- The relationships between HVA and NE metabolites can be exploited to calculate the dopaminergic component of total HVA measurement in plasma and urine. This strategy has been suggested by recent primate studies and needs to be replicated and developed further in humans. If successful, this strategy may prove to be an advance in elucidating the role of brain DA neurons in various diseases and in physiologic conditions.

- Since different sets of factors can affect HVA measurements in different body fluids, using more than one body fluid for HVA measurements in clinical studies could enhance their usefulness in providing information about central DA neuronal activity.

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