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COMMENTARY

ORIGIN AND DISTRIBUTION OF 3-METHOXY-4-HYDROXYPHENYLGLYCOL IN BODY FLUIDS

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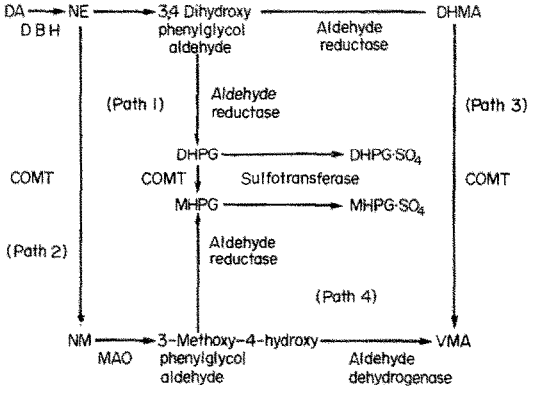
Central noradrenergic neuronal systems appear to be involved in affective states. This conclusion was reached after early observations that a number of pharmacological agents which altered mood states, also altered brain amine metabolism. Originally proposed as the "catecholamine hypothesis of affective disorders", the theory postulated that enhanced noradrenergic neurotransmission in brain is associated with an elated mood, whereas depression is associated with decreased norepinephrine (NE) activity [1–3]. While in later years variations of this hypothesis were introduced to include other neurotransmitters, the importance of central noradrenergic activity in manic-depressive illness has remained a main theme. In the discussion to follow, the current state of knowledge concerning NE metabolism will be summarized, and the usefulness of peripheral measurements of the NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) as an index of central NE neuronal activity will be illustrated.

Metabolism of norepinephrine

The possible pathways of NE metabolism are summarized in Fig. 1. The catabolic pathways have been numbered for ease of reference, and each pathway will be discussed separately. All intermediates noted have been isolated from brain tissue [4–6]. The pathways shown can be divided into two groups: those that lead to MHPG-SO_4 (pathways 1 and 2) and those that lead to vanillylmandelic acid (VMA) as an end product (pathways 3 and 4). The paths leading to VMA are in competition with those leading to MHPG-SO_4 where



ig. 1. Proposed pathways of central norepinephrine metabolism.

they coexist. Therefore, assessment of the relative contribution of these paths is necessary for the assessment of the portion of central NE metabolism resulting in MHPG-SO_4 formation.

Formation of vanillylmandelic acid

Early work had suggested that VMA can be formed in the brain from NE. Chase *et al.* [7] found that intraventricularly(ivt.) administered [^{14}C]dopamine (DA) was metabolized predominantly to VMA rather than to MHPG in dog brain. In a similar study, intraventricular administration of [^{3}H]normetanephrine (NM) to rats also led to VMA formation but the major metabolite was MHPG-SO_4 [8]. Karoum *et al.* [9], using pargyline as a monoamine oxidase (MAO) inhibitor and probenecid to block the efflux of MHPG-SO_4, estimated the kinetics of formation and elimination of MHPG-SO_4 and VMA in the rat brain. In that study, endogenous metabolites measured by gas chromatography-mass spectrometry (G.C.-M.S.) revealed a ratio of MHPG to VMA of 50:1. By analyzing the relative rates of metabolite formation and decline following pargyline, Karoum *et al.* concluded that the 50:1 ratio of MHPG to VMA was due to the fact that MHPG was formed fourteen times faster than VMA, and VMA was eliminated four times faster than MHPG.

In a recent study, electrolytic lesions of rat locus coeruleus produced parallel decreases of whole brain NE and MHPG with no changes in VMA [6]. This finding confirmed the previously established MHPG decrease in rat cortex following locus coeruleus lesion [10]. These results bear greater specificity to the NE neuron than the administration of labeled amines or their precursors and suggest that the [^{14}C]VMA formation measured by Chase *et al.* [7] may not be related to NE *per se*. The regional distribution of MHPG and NE has also been shown to differ from that of VMA in rat brain [11] with MHPG being highest in the hypothalamus, while VMA is concentrated in the striatum. In fact, the association of VMA with the striatum has led to the speculation that brain VMA might be formed from DA, or one of its acid metabolites (3,4-dihydroxyphenylacetic acid, HVA), rather than from NE. Finally, the regional distribution of MHPG-SO_4 and NE is the same for both compounds [12]. Taken together, the failure of VMA levels to change following locus coeruleus lesions, the high MHPG to VMA ratio in the brain, and the regional distribution of VMA clearly indicate that VMA is not a major metabolite in the CNS. Since

3,4-dihydroxymandelic acid (DHMA), the other metabolite of pathway 3, is found only in trace quantities in the brain [5], pathways 3 and 4 are not significantly involved in central NE catabolism.

Formation of MHPG and DHPG

MHPG is a major NE metabolite in the brain of rats, guinea pigs, cats, monkeys [13], dogs [14] and rabbits [15]. Where studied, 3,4-dihydroxyphenylglycol (DHPG) was also identified as a major metabolite. Both MHPG and DHPG are subject to sulfate ester formation, which occurs to varying degrees under different conditions and is largely species dependent. The formation of such conjugates will be considered in another section. Two separate biochemical pathways lead to MHPG formation (pathways 1 and 2). In rat brain, MHPG plus DHPG constitute 80 per cent of central NE catabolism [16] with MHPG accounting for between 20 per cent [17] and 50 per cent [16] of this total. Early attempts to differentiate between pathways 1 and 2 involved intraventricular administration of labeled intermediates and subsequent identification of metabolites by thin-layer chromatography. Using such an approach, Mannarino *et al.* [18] found only trace amounts of [^{14}C]DHPG in cat brain following ivt. administered [^{14}C]NE. Similarly, Schanberg *et al.* [8] reported a low DHPG to MHPG ratio in rat brain following ivt. administered [^{3}H]NM. A slightly higher ratio of DHPG to MHPG (3:5) was found after intraventricular [^{14}C]NE administration by Sugden and Eccelston [19]. While the above reports are somewhat discrepant, they clearly indicate that labeled ivt. administered NE and NM are preferentially recovered as MHPG. In contrast, Rutledge and Jonason [5] found that *in vitro* formation of [^{14}C]DHPG, by incubation of [^{14}C]DA with slices from rabbit cortex, was approximately twice that of MHPG.

Eccleston and Ritchie [20] re examined the DHPG:MHPG ratio in rat brain using intraventricular [^{35}S]sodium sulfate administration. Since both DHPG and MHPG are apparently rapidly sulfate-conjugated in the rat brain, the relative [^{35}S] distribution should reflect the ratio of their formation. Under these conditions, labeled sulfate was found equally distributed between the two catabolites. This result was supported by unity ratios of endogenous DHPG:MHPG, measured by G.C.-M.S., in the hypothalamus of the rabbit, mouse and cat [21]. Eccleston and Ritchie [20] also estimated the rate of MHPG-SO_4 formation by injecting (ivt.) varying amounts of unlabeled MHPG along with labeled sulfate. In addition, they estimated the rates of DHPG-SO_4 and MHPG-SO_4 by injecting (ivt.) DHPG and labeled sulfate. The results showed that the "half-saturating dose" for the formation of [^{35}S]MHPG-SO_4 from added DHPG (5.6 nmoles) was nearly identical to the "half-saturating dose" for the conjugation of exogenously added MHPG (5.1 nmoles). Their results suggested that, when DHPG was added, the sulfotransferase step was rate limiting in the formation of MHPG-SO_4. While the "half-saturating doses" were similar whether exogenous MHPG or DHPG was added, the apparent V_{max} values for [^{35}S]MHPG-SO_4 formation were not. On a molar basis, almost exactly twice as much [^{35}S]MHPG-SO_4 was formed from MHPG as was formed from DHPG per unit time. This indicates that approximately equal amounts of MHPG-SO_4 and DHPG-SO_4 were formed from DHPG. Since this is the same ratio as was obtained when labeled sulfate was given alone, and since any MHPG derived from NM would alter this ratio, the results suggest that most MHPG-SO_4 is formed via pathway 1. However, it will be shown that this pathway may not always be the main route to MHPG formation.

Since MAO is associated with mitochondria and catechol-*O*-methyltransferase (COMT) is largely extraneuronal [22], DHPG formation should be intraneuronal, whereas MHPG should be formed extraneuronally. Such a hypothesis is supported by experiments in which intraneuronal and extraneuronal metabolism was altered by treatment with 6-hydroxydopamine (6-OHDA) or reserpine [23]. When [^{3}H]NE was given ivt. to animals treated previously with 6-OHDA, the formation of [^{3}H]MHPG was *ca.* twice that of [^{3}H]DHPG indicating that, in the absence of presynaptic terminals, catabolism of exogenous NE preferentially proceeds via pathway 2 to form MHPG. In another experiment, this group of investigators first administered [^{3}H]DA ivt. followed by reserpine (i.p.). In this case, intraneuronally released [^{3}H]NE resulted in a 2-fold increase in [^{3}H]DHPG compared to [^{3}H]MHPG. Preferential formation of MHPG over DHPG when NE is predominantly metabolized extraneuronally was illustrated after intraventricular [^{3}H]tyrosine administration to rats injected chronically with desipramine (DMI) [24]. Since DMI blocks NE uptake, the metabolites formed under these conditions should reflect extraneuronal catabolism. In the above study, chronic DMI treatment significantly reduced [^{3}H]DHPG levels while [^{3}H]MHPG formation was not greatly affected. This is contrary to the view that MHPG is formed from DHPG via pathway 1, but rather supports the interpretation that, under conditions of reuptake blockade, most MHPG is formed extraneuronally from NM (pathway 2).

The intraneuronal or extraneuronal site of MHPG formation has also been studied by analysis of the subcellular localization of the participating enzymes. Anderson *et al.* [25] isolated two forms of aldehyde reductase from rat brain. One form, primarily associated with mitochondria, had a high affinity for both NADH and NADPH and was not inhibited appreciably by pentobarbital of 5-hydroxyindoleacetic acid (5-HIAA). The second enzyme form was localized primarily in the cytosol, had a low affinity for NADH, a higher affinity for NADPH, and was inihibited by pentobarbital and 5-HIAA. Anderson *et al.* studied the formation of MHPG by brain homogenates in the presence of various combinations of these cofactors and inhibitors. They showed that *ca.* 70 per cent of aldehyde reductase active in MHPG synthesis was of mitochondrial origin, indicating that this enzymatic reaction probably occurs intraneuronally. An intraneuronal location for MHPG formation has also been demonstrated *in vivo*. Peterson and Sparber [26] found that intraventricular [^{3}H]NE administration resulted in a greater increase in [^{3}H]NM than [^{3}H]MHPG in *d*-amphetamine-treated rats. Since *d*-amphetamine can act as an uptake blocker, a greater increase in [^{3}H]NM, which is formed extraneuronally, than [^{3}H]MHPG would be expected if MHPG formation was primarily

intraneuronal. Since the pathways of NE reuptake and NM formation are in competition for exogenous NE, the data imply that conversion of extraneuronal NE to NM must be about as rapid as reuptake for significant amounts of NM to have been formed. The fact that [^{3}H]NM accumulated with *d*-amphetamine treatment suggests that this agent may be an effective inhibitor of both NM and NE uptake. Hendley *et al.* [27] have shown that under normal circumstances NM uptake in brain slices is extremely rapid, and, in fact, much more rapid than NE uptake. In the same study, it was shown that accumulated NM, unlike NE, remained in the cytosol and was not bound to a particulate fraction, so that it could be readily available to intraneuronal MAO.

The foregoing discussion demonstrates that MHPG can be formed via either pathway 1 or 2 and that both intraneuronal and extraneuronal formation is possible. The question then should not be which pathway leads to MHPG formation, but rather, under what circumstances will a particular pathway predominate? The finding of a unity ratio of DHPG:MHPG by G.C.-M.S. [21] and by intraventricular administration of labeled sulfate [20] suggests that under "baseline conditions" most released NE will be reaccumulated and subsequently metabolized via pathway 1. However, the balance between NE reuptake and NM formation must be relatively unstable. This is supported by the fact that reserpine-induced intraneuronal metabolism favors DHPG, rather than a 1:1 ratio [23]. If intraneuronal metabolism does, in fact, favor DHPG formation, *ca.* 50 per cent of released NE must be metabolized to NM in order to maintain a 1:1 ratio of DHPG to MHPG. The tenuous balance between NE reuptake and NM formation is also evident in experiments in which labeled NE was given ivt. [18, 19]. In these experiments, exogenous labeled NE resulted in a shift favoring pathway 2 because it apparently saturated the uptake system. Similar shifts were observed in experiments in which uptake was blocked with 6-OHDA [23], with DMI [24], or with *d*-amphetamine [26]. Therefore, it appears that a greater portion of released NE will end up as MHPG under conditions of increased NE availability or uptake blockade, and one might predict that MHPG levels might be more sensitive indicators of increased rather than decreased turnover.

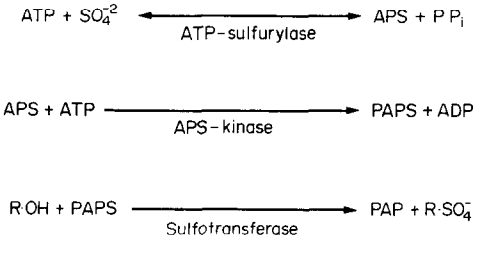


Fig. 2. Scheme for formation of "active sulfate" with subsequent sulfate esterification. Abbreviations used: ATP, adenosine triphosphate; APS, adenosine-5′-sulfatophosphate; PAPS, 3′-phosphoadenosine,5′-phosphosulfate; R·OH, either 3-methoxy-4-hydroxyphenylglycol (MHPG) or 3,4-dihydrophenylglycol (DHPG). Adapted from Eccleston and Ritchie [20].

Thus, it may not be a coincidence that more consistent findings have been obtained in manic rather than depressive states with regard to plasma or urinary MHPG levels [28, 29]. What is less clear is whether MHPG formation from NM occurs intraneuronally or extraneuronally. The fact that DMI treatment had no effect on MHPG formation [24], whereas *d*-amphetamine treatment resulted in accumulation of NM, suggests that *d*-amphetamine might inhibit NM uptake and that DMI might not. If this were the case, we might conclude that NM is normally rapidly accumulated by presynaptic terminals and subsequently converted to MHPG through the action of intraneuronal MAO and aldehyde reductase, and that extraneuronal enzymes have only a small part in this conversion. Unfortunately, we know of no study which has specifically addressed this question.

Formation of sulfate esters

Eccleston and Ritchie [20] described the reactions leading to the formation of conjugated MHPG and DHPG (Fig. 2). In summary, an "activated sulfate" is formed in a two-step reaction at the expense of two ATP molecules. This activated sulfate is then added to the appropriate glycol by means of a sulfotransferase to form the sulfate ester. The sites of ATP-sulfurylase, adenosine 5′-sulfatophosphate (APS)-kinase and sulfotransferase are unknown. However, all the enzymes necessary for conjugate formation must be present in the brain, since sulfate esters of MHPG can be synthesized from inorganic sulfate *in vitro* using brain homogenates [30]. Within the brain, the parallel regional distribution of NE and MHPG-SO_4 suggests that sulfation occurs in close anatomical proximity to the noradrenergic neuron [31, 32]. Less is known about the subcellular distribution of the sulfate conjugating enzymes. However, at least the sulfurylase must be extraneuronal since intraventricular administration of [^{35}S]sodium sulfate leads to ester formation [20], and it is known that nerve-endings are impermeable to sodium sulfate [33].

Sulfation may serve to detoxify MHPG in analogy to hepatic detoxification of phenolic compounds [20, 34], since only the sulfate of MHPG is actively transported across the blood-brain barrier [17]. On the other hand, free MHPG diffusion across cell membranes is essentially unrestricted [35]. Large species variability with respect to the effectiveness of central MHPG sulfation, however, argues against such a primary detoxicant role (see Table 1). While sulfation may serve the detoxification and/or transport of other metabolites [30, 37], MHPG sulfation may be fortuitous and due to a lack of specificity of the conjugating enzymes. In fact, Ken-

Table 1. Chemical form of brain MHPG

Species	% Conjugated	Ref.
Rat	100	13
Guinea pig	100	13
Cat	16	13
Monkey (rhesus)	6	13
Monkey (green)	73	13
Man	25	36

nedy [38] suggested that sulfation plays a major role in the inactivation of neurotransmitters in lower organisms, in which MAO and COMT are absent. It is possible that sulfation has been phylogenetically retained in higher organisms as a general mechanism of detoxification from a time when it served a more central role. Even though the exact role of MHPG sulfation is unclear, the importance of such conjugates should not be underestimated. As will be shown in the following section, the presence of MHPG conjugates has been advantageous in the evaluation of MHPG as an index of NE turnover.

MHPG as an index of NE turnover

MHPG is a reliable index of central NE turnover as indicated by (1) measurement of NE metabolites after metabolic manipulation, (2) measurement of MHPG following drugs that affect noradrenergic neurons, (3) measurement of MHPG after lesion or stimulation of the locus coeruleus, and (4) measurement of MHPG following experimentally induced stress which increases NE activity.

Early experiments in laboratory animals demonstrated that the rates of formation of serotonin and dopamine could easily be estimated by assay of their acid metabolites [39, 40]. These metabolites are eliminated by a probenecid-sensitive transport system. If efflux is blocked by probenecid, the metabolites accumulate in the brain at a rate proportional to their rate of formation and this should reflect the overall turnover rate. Using this method, Meek and Neff [17] estimated the rate of MHPG-SO_4 formation and evaluated it as an index of NE turnover in the rat brain. For comparison, the rate of elimination of MHPG-SO_4 after inhibition of NE breakdown with pargyline, which decreased MAO activity by 98 per cent, was also measured. Theoretically, the rate of elimination should be the same as the rate of formation since the two processes must be in equilibrium to maintain a steady state MHPG-SO_4 level. However, in the above study, the rate of MHPG-SO_4 formation (244 pmoles/g/hr) was significantly greater than the rate of elimination (130 pmoles/g/hr). To explain this discrepancy, Meek and Neff suggested that, in spite of pargyline treatment, endogenous stores of unidentified MHPG precursors may have continued to form MHPG-SO_4 which would have effectively decreased the apparent rate of elimination in their experiments. However, since very little MHPG is normally formed from DHPG (as discussed earlier) and since nearly all rat brain MHPG is in the sulfate form [13], this interpretation is unlikely. It was also suggested that probenecid treatment may have accelerated NE turnover which resulted in an overestimate of the rate of MHPG-SO_4 formation. This is much more likely, since Stone [41] reported that probenecid increased NE turnover *ca.* 80 per cent in the rat hypothalamus even though he administered one half the dose used by Meek and Neff [17]. Since the latter investigators found the rate of formation to be 88 per cent higher than the rate of elimination, probenecid-induced stimulation of NE turnover explains the discrepancy between the two measures of turnover used in the above cited study.

Stone [41] took a somewhat different approach to estimate NE turnover. In his study in rat hypothalamus, [^{3}H]NE synthesis from ivt. administered [^{3}H]tyrosine was compared with [^{35}S] MHPG-SO_4 from ivt. administered [^{35}S]Na_2SO_4. To evaluate the relative sensitivity of these two indices, Stone used drugs that alter noradrenergic activity. While the α-adrenergic agonist clonidine significantly decreased both [^{3}H]NE synthesis and [^{35}S]MHPG-SO_4 formation, the α-adrenergic receptor blocker phenoxybenzamine increased both [^{3}H]NE and [^{35}S]MHPG-SO_4 levels. The turnover changes estimated by these two independent methods were in good quantitative as well as qualitative agreement. Other pharmacological manipulations have lent further support to the link between MHPG-SO_4 formation and NE turnover. A number of receptor blockers enhance both NE and DA turnover in the CNS [42]. Methiothepin, haloperidol, clozapine, thioridazine, chlorpromazine and pimozide increased MHPG-SO_4 to varying degrees due to the feedback activation of NE neurons secondary to receptor blockade [43].

Direct evidence supporting the correlation of MHPG levels with NE activity was obtained from lesions or stimulation of the rat nucleus locus coeruleus which contains a high density of NE neurons [44, 45]. Lesions produced a reduction in total MHPG proportional to the degree of destruction [10, 46], while stimulation significantly increased MHPG levels [47, 48].

Several types of "stress" have been correlated with increased noradrenergic activity [49, 50]. Electric foot shock increased both NE turnover and MHPG-SO_4 levels in the rat [46, 51]. No increase in MHPG was found when locus coeruleus lesioned rats were stressed, but a 59 per cent increase in MHPG levels was measured in the cortex of stressed animals which had received sham lesions [48]. Similarly, cold stress increased MHPG by 50 per cent in mouse brain [52] and by 19 per cent in rat brain [17]. Stone [53] found an 18 per cent increase in rat brain MHPG following exhaustive running stress. Taken together, the above experimental approaches clearly demonstrate that levels of MHPG are a valid index of central NE activity.

Appearance of MHPG in body fluids

The techniques utilized in the above animal studies are not applicable to studies in humans. Autopsy specimens are not readily available, and the problem of postmortem changes has not been resolved. Therefore, considerable work has focused on the measurement of MHPG in body fluids. For such measures to be valid, a relatively large fraction of the metabolite must be of central origin. In addition, changes in central NE activity must result in measureable changes in MHPG levels in the fluid sampled. A number of such measurements have been performed using cerebrospinal fluid (CSF), urine, and blood from laboratory animals and humans.

Measurement of HVA, DOPAC and 5-HIAA in CSF following probenecid is a valuable approach in the assessment of central dopamine and serotonin turnover [54–56]. Since the transport of MHPG-SO_4, but not of free MHPG, across the blood-brain barrier is also probenecid sensitive, this technique was utilized to assess NE turnover. Levels of MHPG-SO_4 in cisternal CSF of the rabbit increase following probenecid [15]. The brain origin of MHPG in CSF was demonstrated by a ventricular-cisternal gradient of 5 (v/c) for MHPG-SO_4 in the dog [14]. Such a gradient would not

be expected if the spinal cord made a large contribution to MHPG levels in CSF. However, attempts to utilize the probenecid technique with monkeys and humans were unsuccessful because in simian and human CSF MHPG is primarily in the unconjugated form [57, 58] which renders probenecid ineffective. As expected, no increase in MHPG was found following probenecid either in monkey [59] or in man [58, 60], in spite of the fact that probenecid may have actually increased NE turnover (as discussed in the previous section).

It now appears that the probenecid technique is of little use in human studies. Newer evidence questions the brain origin of CSF MHPG in man. Unlike the dog [14], no gradient of MHPG, conjugated or free, was found between ventricular and lumbar CSF in monkey [61] or in man [58, 62, 63], even though a recent report indicated that a small MHPG gradient may be present in human CSF [64]. The absence of a significant ventricular-lumbar gradient was further demonstrated in patients with spinal cord transections and blockade of CSF flow [65–67]. These results suggest that MHPG in human CSF may be largely derived from spinal cord metabolism. Kessler *et al.* [35, 59], using subarachnoid perfusion with artificial CSF followed by serial sectioning of the spinal cord, measured the capillary exchange rate of MHPG in monkeys. They concluded that MHPG was rapidly and continuously lost through capillaries within the tissue, and that for this reason only a fraction of the total is reflected in the CSF. Furthermore, since unconjugated MHPG freely passes through cell membranes, and since brain MHPG is primarily unconjugated in man [36, 68], the surrounding tissue would act as a large exchangeable pool and tend to "buffer" fluctuations in MHPG levels resulting from changes in NE turnover [35, 59]. This buffering effect is illustrated in the absence of circadian rhythm in vertricular CSF MHPG even though NE rhythms have been identified in this body fluid [69]. In conclusion, CSF MHPG reflects predominately spinal cord metabolism of NE and is slow in reflecting changes in brain NE turnover. In spite of the aforementioned difficulties, altered CSF MHPG levels have been correlated with affective disorders in man [62, 70–72], although the significance of these correlations is not unequivocal [73].

Urine is the most thoroughly studied body fluid with respect to MHPG excretion. Evaluation of how closely urinary MHPG reflects CNS metabolism has attracted so much attention that numerous attempts, using diverse techniques, have been directed to this problem [74–77]. Most estimates agree that at least 30–50 per cent of MHPG found in urine is of central origin and that urinary NM and VMA are almost exclusively derived from peripheral NE metabolism [78–81]. Human experiments, using isotopic dilution techniques, indicate that a much higher perpercentage (80 per cent) of urinary MHPG may be of central origin in man [81]. Nevertheless, a number of conflicting reports have appeared. Chief among these is the controversy over the effects of central sympathectomy, using ivt. administered 6-OHDA, on urinary MHPG levels. If a high proportion of urinary MHPG is indeed of central origin, such a lesion would result in decreased urinary MHPG levels. Such a decrease was indeed found by Maas *et al.* [82], but not by Breese *et al.* [83] in the monkey, although the latter group did find a significant decrease in 6-OHDA lesioned rats. Since failure to find decreased urinary MHPG following chemical lesioning might indicate that urinary MHPG is of peripheral origin, several investigators replicated these experiments under blockade of peripheral NE metabolism. Bareggi *et al.* [84] and Karoum *et al.* [85] treated control and 6-OHDA animals with the peripheral MAO inhibitor debrisoquine (DB). This treatment resulted in a 50–70 per cent decrease in urinary MHPG, in accord with earlier estimates that 25–50 per cent of MHPG in rat urine is of central origin. Bareggi *et al.* [84] found that ivt. administered 6-OHDA failed to decrease MHPG levels in 24-hr urines from DB-treated animals, whereas Karoum *et al* [85] found a significant decrease under similar conditions but using 7-hr urine collections. The latter study also included lesioned animals without DB treatment with both 7-hr and 24-hr urine collections. Chemical lesioning decreased the 7-hr urinary MHPG but had no effect on the 24-hr MHPG level. Furthermore, MHPG levels in untreated controls were significantly higher in 24-hr urine collections than were levels obtained by extrapolation of 7-hr to 24-hr data. These results suggest that the effect of 6-OHDA lesioning is masked by diurnal variations when 24-hr collections are used. Since 6-OHDA had no effect when 24-hr samples were compared, one must assume that a large portion of overnight MHPG excretion is of peripheral origin. However, some caution must be exercised in attempting to extrapolate these results to man, where the CNS is believed to contribute a significantly larger portion of total MHPG in urine.

A circadian rhythm in urinary MHPG levels has been found in man [86, 87]. Cymerman and Francesconi [86] compared the effects of moderate cold stress on diurnal patterns of urinary MHPG and VMA. Cold stress altered the periodicities of both MHPG and VMA cycles; whereas MHPG levels increased, VMA levels did not. The increase in MHPG was due to the combined effects of an elevated baseline, as well as an increase in the amplitude of the diurnal cycles. The finding that MHPG levels increased whereas VMA levels did not, suggests that these metabolites do not share a common origin. Since VMA is the principal peripheral NE metabolite, the data support the view that most urinary MHPG is of central origin. In addition, the fact that cold stress increased the amplitude of MHPG cycles further suggests that diurnal variations, as well as baseline levels, are of central origin. Although the diurnal cycle is a potential confounding factor, extensive clinical literature has demonstrated the usefulness of urinary MHPG in the study of affective disorders. However, discussion of these findings is beyond the scope of this communication, and the interested reader is referred to several informative reviews [88–90].

Very few studies have reported on plasma MHPG levels. Plasma levels are low and the volume of plasma available for analysis is by necessity much less than that of urine. In addition, CSF and urinary methods are ill-suited for routine measurements of plasma samples. These problems have now been largely overcome with the advent of appropriate methodology [91], and it is

expected that the future will see an increased popularity of plasma measurements.

MHPG in blood is compartmented between plasma and red cells with the latter containing *ca.* 25 per cent of the total [92]. About 35 per cent of plasma MHPG is in the free form and the remainder is conjugated as a sulfate or glucuronide, in both simian and human blood [92]. Plasma MHPG levels, like those of CSF and urine, reflect changes in noradrenergic activity. A direct relation has been reported between stimulation of the locus coeruleus and increases in plasma MHPG in the rat [93, 94]. In addition, clinical studies have now demonstrated correlations between plasma MHPG levels, affective disorders, and the effects of psychotropic compounds [95–98].

Since the routine measurement of plasma MHPG is a relatively recent development, few attempts have been made to establish what percentage of blood MHPG is derived from brain. Using the relative enrichment of D_3 in MHPG-SO_4 following MHPG-D_3 administration, Blombery *et al.* [99] suggested recently that 10–39 per cent plasma MHPG-SO_4 may be derived from the brain in monkeys. However, since MHPG occurs predominantly in the unconjugated form in human and simian brain [36, 100], these results most probably reflect the relative contributions of the hepatic sulfate and glucuronide conjugations and are thus not likely to show the specific origin of the MHPG conjugate. A relatively high proportion of blood MHPG may be derived from central origin. As shown above, in man as much as 80 per cent of urinary MHPG may be derived from central NE, according to some estimates. Since urine is a filtrate of blood, this percentage should be at least as high as in urine. More direct evidence was obtained when the rate of MHPG excretion from brain into blood was measured. Maas *et al.* [100], assessing the difference between arterial and venous MHPG levels obtained from the jugular bulb or sigmoid sinus, demonstrated a significant venoarterial difference in thirteen monkeys. Using the same technique and in conjunction with the administration of clonidine, they showed that the noradrenergic agonist caused an 80 per cent decrease in the rate of MHPG excretion into blood. Conversely, the noradrenergic antagonist piperoxan increased MHPG excretion up to 718 per cent. Assuming that MHPG is not significantly further metabolized, this group contrasted urinary excretion rates with brain production, and concluded that a substantial portion of total body MHPG production is derived from brain. These results have now been verified in man, and current estimates indicate that at least 64 per cent of total MHPG production is due to brain metabolism [101].

Plasma MHPG levels, like those of urine, exhibit a diurnal rhythm. Markianos and Beckmann [102] showed that plasma MHPG levels are maximal during the morning and early afternoon, and are minimal in the late evening and overnight. This pattern is similar to that reported for urinary MHPG [86, 87] and it is probable that MHPG levels in both fluids reflect fluctuations in the same pool. Evidence has been given above which supports the assumption that diurnal urinary MHPG changes are of central origin. However, Markianos and Beckman [102] found that MHPG cycles were very closely correlated with cycles of serum dopamine-β-hydroxylase (DBH) activity, an enzyme which is almost exclusively of peripheral origin. This finding suggests that both plasma MHPG and DBH may originate in peripheral neurones. There is evidence which can explain this apparent paradox. Perlow *et al.* [69] have shown the presence of a circadian rhythm of NE in ventricular CSF from rhesus monkeys. This pattern is quite similar to the patterns reported for plasma and urinary MHPG as well as serum DBH in man, and suggests that circadian cycles of NE metabolism in the central and peripheral nervous system are in phase.

Conclusions

Current evidence indicates that the measurement of MHPG levels in brain or body fluids is a sensitive index of central NE turnover. However, there are indications that these levels may be more sensitive to increases than to decreases in turnover. The sensitivity of MHPG in body fluids to changes in CNS activity stems from the fact that in man most total body MHPG is of central origin. This trait is shared with other higher primates but is not evident in laboratory animals, e.g. the rat, where as much as 75 per cent of total MHPG may be of peripheral origin. Some body fluids appear to be more suitable than others. Measurements of MHPG in blood or urine are more specific in reflecting changes in central NE activity than are measurements from CSF. The interpretation of MHPG levels in both blood and urine is complicated by the presence of diurnal variations. Central and peripheral NE activities appear to cycle in unison. Centrally formed MHPG dominates the diurnal cycle in man and higher primates, whereas peripherally formed MHPG dominates the rat diurnal cycle.

A number of studies have shown that manic states can be distinguished from depressive states on the basis of MHPG levels. However, the more important distinction between normal controls and various types of depressive disorders has been more elusive. Recent evidence suggests that such a distinction may be possible through measurement of disturbances of diurnal MHPG cycles, which appear to be different in depressed patients [103]. Peripheral circadian cycles may also be altered in some affective disorders. Van Cauter and Mendlewicz [104] have shown that diurnal patterns in serum DBH activity were altered in bipolar but not unipolar depressives. It will be interesting to see if a similar distinction can be made using MHPG as a parameter. The finding of abnormal peripheral, as well as central, diurnal patterns in depressives permits speculation about the nature of these abnormalities. It is possible that both changes reflect the disturbance of a common clock mechanism (Zeitgeber). Such an oscillator is thought to reside in the suprachiasmatic nucleus, which may regulate a number of diurnal phenomena [105–111] some of which have themselves been implicated in mood disorders [112–116]. If such links can be established, they may well serve to integrate what now appears to be a collection of disjointed and loosely related theories of affective disorders.

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