in the hypothalamus and higher in the NI pituitary during the day; and (ii) deprivation of both food and water, or of water only, increases daytime levels of hypothalamic immunoreactive dynorphin, whereas deprivation of water alone decreases NI pituitary immunoreactive dynorphin.

In view of the relatively rapid catabolism of dynorphin (*1*), increased levels of immunoreactive dynorphin probably reflect increased synthesis and storage, and decreased levels in the NI pituitary probably reflect rapid utilization. There seems to be a reciprocal relation between the levels of immunoreactive dynorphin in the hypothalamus and the NI pituitary, one being high when the other is low.

These results, showing a relation between circadian rhythm and immunoreactive dynorphin, lead to a number of hypotheses concerning the role of dynorphin, including those involving such variables as sleep and general activity, and the possibility that the endorphins may be integral to a biological clock. An immunohistochemical study (*2*) indicates the presence of dynorphin cells in the region of the suprachiasmatic nuclei of the hypothalamus, an area whose destruction disrupts circadian rhythm (*8*). The changes of immunoreactive dynorphin with circadian rhythm suggest that dynorphin may be related to events sensitive to opioid antagonists showing circadian rhythms. Evidence indicating that dynorphin is an endogenous ligand for the κ-opioid receptor (*9*), together with our results, lead to the hypothesis that κ-opioid drugs would be potent in affecting behavioral regulation of ingestion or of circadian rhythms. The interaction among water balance, circadian rhythm, and immunoreactive dynorphin implicates dynorphin in behavioral maintenance of homeostasis.

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4. Standard cages of plastic and wire mesh (55 by 34 by 18 cm) were kept in a colony room at 24°C and 45 percent humidity. The lighting cycle was 12 hours of light and 12 hours of darkness, with lights on at 0800.
5. The goal of the procedures of habituation and handling was to minimize extraneous sources of stress, leaving only those associated with privation as the critical variable. Accordingly, rats were housed in groups to minimize isolation stress. Assays were carried out only after rats were gaining weight across days, indicating that they were habituated to the living conditions [see H. Steinberg, in *Factors Affecting the Action of Narcotics*, M. L. Adler, L. Manara, R. Samanin, Eds. (Raven, New York, 1979), pp. 595–611]. The rats were eating and drinking most of their food and water at night (> 82 percent of each), indicating that they were entrained to the lighting cycle. They were not defecating when being handled and weighed, indicating that they were accustomed to being handled. In addition, only nine rats of each group were killed, so that the last rat left in a cage (having an unusual potential for stress) was not part of the sample.
6. Peptides were extracted by placing tissue in 0.1*N* HCl at 95°C for 10 minutes, and then homogenized and centrifuged at 140,000*g* for 30 minutes at 4°C. The radioimmunoassay, conducted in triplicate, involved adding to plastic tubes (i) 50 μl of extract of the tissue, (ii) 300 μl of buffer, (iii) 100 μl of antiserum solution (dilution, 1:50,000), and (iv) 50 μl of iodinated tracer solution. After an incubation period of 20 hours, unbound ^{125}I-labeled tracer [dynorphin-(1–17)] was removed by charcoal separation.
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Relation Between Plasma and Cerebrospinal Fluid Levels of 3-Methoxy-4-Hydroxyphenylglycol

Abstract. *Concentrations of free 3-methoxy-4-hydroxyphenylglycol in the plasma and cerebrospinal fluid are highly correlated, but concentrations in the cerebrospinal fluid are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from a tumor of the adrenal medulla. This is explained by considering the plasma and cerebrospinal fluid as a two-compartment system in which the rate constants for entry into and exit from the cerebrospinal fluid compartment are similar. 3-Methoxy-4-hydroxyphenylglycol that is synthesized, but not catabolized, in the central nervous system maintains cerebrospinal fluid levels at an increment over those in plasma. This increment can be used to provide the best available index of formation of 3-methoxy-4-hydroxyphenylglycol in the central nervous system.*

In the brain of most species, 3-methoxy-4-hydroxyphenylglycol (MHPG) is the major metabolite of norepinephrine (*1*). Although most MHPG is excreted as a conjugate, about one-third of the total MHPG in human plasma is unconjugated and almost all MHPG in cerebrospinal fluid (CSF) occurs in the free form (*2*). Measurement of CSF concentrations of MHPG has become a widely accepted means of assessing norepinephrine formation and utilization in the central nervous system of man and other primates (*3*). Although in humans (*4*) and monkeys (*5*) there is a highly significant correlation between CSF and plasma levels of MHPG, it has been presumed that both reflect central noradrenergic activity (*5*). From the results obtained in our present study it becomes clear that a substantial portion of free MHPG in human CSF is derived from plasma, that this is to be expected on the basis of kinetic considerations, and that CSF levels of MHPG can be interpreted as reflecting central nervous system norepinephrine metabolism only when the plasma MHPG contribution can be adequately assessed.

To determine the extent to which peripherally formed MHPG can affect CSF levels of this metabolite, we examined plasma and CSF levels of MHPG in patients with phaeochromocytoma, a tumor of the adrenal medulla which contains high concentrations of MHPG (*6*). These patients were expected to have relatively constant high levels of plasma MHPG. Normal subjects and patients with idiopathic orthostatic hypotension (known to have deficits in peripheral

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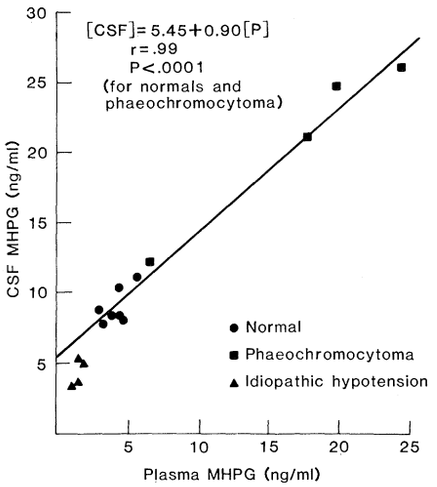

Fig. 1. Relation between concentrations of MHPG in the plasma and in the CSF of normal subjects and patients with phaeochromocytoma or idiopathic orthostatic hypotension.

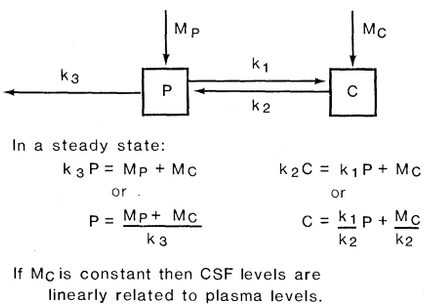

Fig. 2. A two-compartment system representing unconjugated MHPG in plasma and CSF. In this system $[P]$ is the plasma MHPG; $[C]$ is the CSF content; M_p and M_c are the rates of MHPG production in peripheral tissues and central nervous system, respectively; k_1 and k_2 are rate constants for the transfer of MHPG from plasma into CSF and vice versa; and k_3 is the sum of the rate constants for removal of free MHPG plasma by excretion, oxidation to VMA, and conjugation.

norepinephrine secretion) were also included in this study. Blood was collected into plastic tubes containing an anticoagulant, immediately centrifuged in the cold, and the plasma was removed and frozen. We obtained CSF by lumbar puncture from the same subjects, usually within a few minutes of obtaining the blood. Plasma and CSF were kept frozen at − 80°C until we assayed them for MHPG using deuterated internal standards and gas chromatography–mass spectrometry as described (*7*).

We found a close correlation ($r = .998$, $P < .0001$) between plasma and CSF levels of MHPG in seven normal subjects and four patients with phaeochromocytoma (Fig. 1). The regression line has a slope indicating that the CSF level increases 0.90 unit for each unit of plasma MHPG. The intercept is not at the origin; at all plasma concentrations the CSF levels are about 5 ng/ml higher than plasma levels. Both the plasma and CSF levels of MHPG are abnormally low in the patients with orthostatic hypotension, but the CSF levels are even lower than might be expected on the basis of the relation between levels of MHPG in the plasma and CSF of subjects without a known central nervous system disorder.

The close relation between the free MHPG in the plasma and CSF of normal subjects and in patients with phaeochromocytoma indicates that plasma MHPG concentrations influence those in CSF. This is readily explained by kinetic considerations. Plasma and CSF may be viewed as a simple two-compartment system (*8*). The steady-state levels of MHPG in plasma and CSF are maintained by formation of MHPG in the peripheral tissues (at a rate M_p) and in the central nervous system (at a rate M_c). As represented in Fig. 2, M_p enters plasma whereas M_c enters CSF. MHPG is a nonpolar compound that diffuses as rapidly as water out of, and probably into, nervous tissue (*9*). There is no active transport process for secretion of free MHPG into plasma. Because of bulk flow (*8*), the rate constant for exit of MHPG to plasma from CSF, k_2, is probably slightly greater than that for entry of MHPG to CSF from plasma, k_1. As shown in Fig. 2, the rate of entry of free MHPG into the CSF equals the rate of its exit, that is, $k_2 [C] = k_1 [P] + M_c$. This equation can be rearranged to show that the MHPG concentration in CSF, $[C]$, is related to that in plasma; $[C] = (k_1/k_2) [P] + M_c/k_2$. If formation of MHPG in the central nervous system, M_c, remains constant, the relation is a straight line with a slope determined by k_1/k_2. The slope is expected to be close to unity since, as indicated above, k_2 is probably only slightly greater than k_1.

Plasma concentrations of free MHPG are determined by the total rate of MHPG production ($M_p + M_c$) and the sum of the rates of conjugation, metabolism to vanillylmandelic acid (VMA), and excretion, which are combined in a single rate constant (k_3) in Fig. 2. If plasma MHPG levels are increased because of an increase in MHPG production in peripheral tissues, there will be an almost equal increase in CSF levels, provided formation of MHPG in the central nervous system is unchanged (*10*). If formation of MHPG in the central nervous system increases, plasma MHPG will also increase, but the increase in MHPG in the CSF will be greater than that in plasma; the difference between CSF and 0.9 times the plasma MHPG levels ($= M_c/k_2$) will increase (k_2 is probably dependent on diffusion and bulk flow and remains relatively constant). Similarly, with a decrease in central nervous system MHPG production, there will be a greater decrease in CSF than in plasma levels and the difference will decrease.

The data shown in Fig. 1 substantiate the conclusions of this theoretical relation between plasma and CSF levels of MHPG in humans. The difference in CSF and plasma MHPG levels in patients with phaeochromocytoma and in normal subjects is similar (about 4.5 ng/ml). This is equivalent to M_c/k_2, and is similar in the patients and normal subjects because the high CSF levels of MHPG in patients are not due to production for MHPG in the central nervous system. Patients with idiopathic orthostatic hypotension have lesions that include the intermediolateral columns of the spinal cord and suffer from deficient peripheral sympathetic neuronal function. These deficiencies account for the low concentrations of MHPG in the plasma and CSF. The low plasma levels in these patients may be attributed largely to the peripheral deficit. The central nervous system lesions of areas normally containing norepinephrine is reflected in the corrected difference ($[C] - 0.9 [P]$) between CSF and plasma MHPG levels. In patients with orthostatic hypotension, this difference is only 2.5 ± 0.2 ng/ml, significantly ($P < .01$) lower than in the four patients with phaeochromocytoma (5.7 ± 0.7) or seven normal subjects (5.2 ± 0.5).

To our knowledge only one previous study addressed the question of entry of plasma MHPG into human CSF. Chase *et al.* (*11*) infused ^{14}C-labeled MHPG intravenously for 15 minutes and examined plasma and CSF radioactivity at various times after the infusion. They found that the peak amount of ^{14}C in CSF (which occurred 2 to 4 hours after the administration of the labeled compound) was only about 6 percent that of the ^{14}C in plasma at the end of the 15-minute infusion and concluded that there was poor penetrance of MHPG from plasma into CSF. At that time it was not realized that MHPG is rapidly metabolized to its conjugates and to VMA (*13*) and that the amount of free ^{14}C-labeled MHPG in CSF may have been equal to, or even higher than, the level in plasma at the time of removal of the CSF.

Studies in animals in which MHPG is conjugated in brain are not valid models

for humans since the conjugate is transported rapidly out of brain ($k_2 >> k_1$). The ratio of k_1 to k_2 is small so that the levels of free and conjugated MHPG in brain are dependent mainly on their formation in brain (M_c/k_2). Since monkeys form little or no conjugates of MHPG in brain, studies in these animals are valid models for humans. Ellsworth *et al.* (*5*) showed that in monkeys CSF concentrations of MHPG are proportional, but always higher, than plasma concentrations. In their study, the slope of the best-fit line for 67 matching plasma and CSF samples was close to unity; the difference between CSF and plasma MHPG concentrations was relatively constant (about 20 ng/ml). Perhaps MHPG is formed more rapidly per unit volume of CSF in monkeys than in man, or the larger difference is a result of sampling of CSF from the cisterna magna rather than from the lumbar area. Concentrations of unconjugated MHPG reflect local norepinephrine metabolism only if the equilibrium of CSF (or partition of brain tissue) with plasma MHPG is considered.

As shown above, concentrations of MHPG in the plasma and CSF are dependent on the sum of the rates of MHPG formation in both the central nervous system and the peripheral tissues and the rate of MHPG metabolism. Peripheral sources probably predominate in determining plasma MHPG levels. Neither plasma levels nor urinary excretion of MHPG are valid indices of brain norepinephrine metabolism since MHPG from brain appears to account for only about 30 percent of the total body production of MHPG (*12*). On the basis of the theoretical considerations and the supporting empirical evidence (for example, in patients with idiopathic orthostatic hypotension), it appears that concentrations of MHPG in human lumbar CSF can provide a valid index of central MHPG production, but only when appropriately corrected by subtracting 90 percent on the plasma MHPG concentrations.

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10. This may be expressed mathematically in terms of the equations that describe the two-component system: $[P] = (M_p + M_c)/k_3$, $d[P]/dM_p = 1/k_3$, and $d[P]/dM_c = 1/k_3$. For the levels in CSF: $[C] = (k_1/k_2 \cdot k_3)M_p + (k_1/k_2 \cdot k_3 + 1/k_2)M_c$, $d[C]/dM_p = (k_1/k_2 \cdot k_3)$, whereas $d[C]/dM_c = k_1/k_2 \cdot k_3 + 1/k_2$. Thus, when M_p changes, $d[C]/d[P] = k_1/k_2$, whereas when M_c changes, $d[C]/d[P] = k_1/k_2 + k_3/k_2$. If both M_p and M_c change, the slope will be intermediate (a weighted mean) between k_1/k_2 and $k_1/k_2 + k_3/k_2$, depending on the relative magnitude of the changes in M_p and M_c.
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Effects of Tyrosine Administration on Serum Biopterin in Normal Controls and Patients with Parkinson's Disease

Abstract. *After administration of tyrosine, total concentration of biopterin, the cofactor for tyrosine hydroxylase, was increased in the striatum, adrenal glands, and serum of rats, and in the serum of humans. Serum biopterin is lower in patients with Parkinson's disease than in normal controls. After oral administration of tyrosine, the increase in serum biopterin concentration was smaller in patients with Parkinson's disease (less than twofold) than in healthy controls (three- to sevenfold). These results suggest that tyrosine may have a regulatory role in biopterin biosynthesis and that patients with Parkinson's disease may have some abnormality in the regulation of biopterin biosynthesis.*

The enzymic hydroxylation of tyrosine, the first step in dopamine biosynthesis, is catalyzed by tyrosine hydroxylase, which requires L-*erythro*-tetrahydrobiopterin (BPH_4) as cofactor. Both tyrosine hydroxylase activity and total biopterin concentration are greatly decreased in the striatum of patients with Parkinson's disease (*1*). Hydroxylase cofactor content in the cerebrospinal fluid of these patients is also decreased in comparison with that of controls (*2*). Since BPH_4 is an essential cofactor for tyrosine hydroxylation, these results suggest that the reduction in dopamine in the parkinsonian striatum may be due to reductions in the concentration of striatal BPH_4 and in tyrosine hydroxylase activity. The concentration of BPH_4 in catecholaminergic neurons may be lower than the value of the Michaelis constant for the cofactor of nonphosphorylated tyrosine hydroxylase, and catecholamine biosynthesis in vivo may be regulated by both BPH_4 concentration and tyrosine hydroxylase phosphorylation (*3*). Reserpine treatment or insulin-induced hypoglycemia, which increases tyrosine hydroxylase (*4, 5*), produced a significant increase in the BPH_4 content in the adrenal medulla in rats (*6, 7*).

Since tyrosine increases catecholamine biosynthesis through the change in tyrosine hydroxylase activity (*8*), we have investigated the effect of tyrosine administration on total biopterin concentration in tissues and serums from rats and from normal human subjects and patients with Parkinson's disease.

Tyrosine (1 g per kilogram of body weight), suspended in 0.2 percent carboxymethylcellulose, was injected intraperitoneally in male Sprague-Dawley rats (200 to 250 g). The rats were decapitated at various time intervals after tyrosine administration; tissues (striatum, adrenal glands, and liver) and blood were immediately removed, and serum was separated. Total biopterin concentration was measured by a newly established, specific, sensitive radioimmunoassay for L-*erythro*-biopterin (*9*). We found that total biopterin concentration was increased 1.5- to threefold in the striatum, adrenal glands, and serum after administration of tyrosine to rats (Fig. 1), but total biopterin content in the liver was slightly decreased. These results suggest that tyrosine may regulate the biosynthesis of biopterin from guanosine triphosphate in central and peripheral catecholaminergic cells.

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