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3-Methoxy-4-Hydroxyphenethyleneglycol Production by Human Brain in vivo

Abstract. *A direct method has been employed to estimate the rate of production by human brain of 3-methoxy-4-hydroxyphenethyleneglycol, the major metabolite of brain norepinephrine, a brain neurotransmitter. Venous specimens were obtained from the internal jugular vein from ten awake human subjects at a puncture site above the common facial vein, the first major source of extracranial inflow. Arterial specimens were simultaneously obtained from the radial artery. Plasma samples were assayed and a highly significant difference was found in the concentration of the metabolite in plasma coming out of the brain (venous blood) as compared to plasma entering the brain (arterial blood). This venous-arterial difference was calculated to be 0.7 ± 0.1 nanogram per milliliter of blood. Assuming an adult brain weight of 1400 grams and normal cerebral blood flow, it is estimated that the rate of production of 3-methoxy-4-hydroxyphenethyleneglycol by the awake human brain is approximately 597 nanograms per minute or 35.8 micrograms per hour. Urine specimens were also collected from six of these subjects during a period of 1 to 3.5 hours, which bracketed the time the blood samples were obtained. For these six subjects the output of 3-methyoxy-4-hydroxyphenethyleneglycol by whole brain was estimated to be 40.9 micrograms per hour, whereas the rate of its excretion into urine was 64.5 micrograms per hour.*

The functioning of brain noradrenergic systems has been related to a variety of pathological states such as obesity, hypertension, depression, and mania (*1*). For these reasons clinical investigators have been particularly interested in the problem of determining the degree to which levels of plasma or urinary 3-methoxy-4-hydroxyphenethyleneglycol (MHPG), the major metabolite of brain norepinephrine (NE) (*2–4*), may reflect the functioning of noradrenergic neurons within the central nervous system (CNS). Previous approaches to this problem have utilized indirect methods; while these studies, in general, suggest that measures of urinary MHPG in larger mammals may provide an index of NE metabolism, there are points of disagreement and several assumptions and inferences are required to reach this conclusion (*3*, *5*). Interest in this problem led to recent studies with monkeys (*Macaca arctoides*), which have shown that there are consistently higher levels of MHPG and other neurotransmitter metabolites, such as homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), in venous blood of the internal jugular bulb than in aortic blood. When these venous-arterial (V-A) differences are multiplied by the rate of cerebral blood flow (CBF) a measure of the rate of production of a particular neurotransmitter metabolite per 100 g of monkey brain per minute can be obtained (*6*). It therefore seemed that one might also be able to measure a difference in neurotransmitter metabolites between internal jugular vein and arterial blood in humans. This proved to be the case, and the initial results of experiments dealing with this question for MHPG are reported here. To our knowledge this is the first demonstration that it is possible to measure in the awake human brain a V-A difference for a neurotransmitter metabolite. The data obtained were also used to estimate the relationship between the rate of production of MHPG by human brain and the quantity of MHPG found in urine.

In this study venous blood samples (10 ml) were obtained from the internal jugular vein of six volunteers at a puncture site 3 to 4 cm above the common facial vein and at the same time samples (14 ml) were taken anaerobically from the radial artery. Similar samples were obtained in four patients in the course of inserting balloon-tipped flotation-direct-

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Table 1. Concentration of MHPG in plasma obtained from the radial artery and the internal jugular vein of human subjects. The venous blood samples were obtained from a puncture site 3 to 4 cm above the common facial vein, which is the first major tributary of the internal jugular vein. The V-A differences are statistically significant ($P < .0005$).

Subject	Concentration of MHPG in plasma (ng/ml)		V-A difference
	Venous	Arterial	
Normal volunteer	5.6	4.9	0.7
Normal volunteer	4.5	4.3	0.2
Normal volunteer	1.9	1.4	0.5
Normal volunteer	2.2	1.8	0.4
Normal volunteer	3.4	2.2	1.2
Normal volunteer	1.9	1.7	0.2
Patient 1	13.2	12.4	0.8
Patient 2	5.8	5.1	0.7
Patient 3	2.2	0.7	1.5
Patient 4	5.1	4.7	0.4
Mean ± S.E.	4.6 ± 1.0	3.9 ± 1.0	0.7 ± 0.1

ed catheters into the pulmonary artery via the internal jugular vein, the right atrium, and the right ventricle. This procedure is routinely performed in certain types of major cardiovascular surgery (*7*). At the point at which the internal jugular vein was punctured—that is, above the common facial vein—there is little contamination of blood coming from the intracranial sources by venous blood draining from extracranial sites (*8*). The volunteers were six healthy subjects, four men and two women, ranging in age from 27 to 57. None of the volunteers had taken any medication within 10 days of the study and all were fully awake and alert during the sampling. Patient 1 (see Table 1) was in septic shock and had received antibiotics, but no other medications, before the internal jugular and radial artery punctures. The other three patients were receiving various combinations of diuretics, coronary artery dilating agents, and medication for the treatment of congestive heart failure. Patient 2 had been treated with a β-adrenergic receptor blocking agent. Patients 2 and 3 received 4 to 12 mg of morphine 2 to 3 hours before the blood specimens were obtained. Patient 4 had been given 10 mg of diazepam 3 hours before the procedure.

The blood was immediately placed on ice and centrifuged and the plasma was removed. As an internal standard, 200 ng of deuterated MHPG per milliliter of plasma was added, and the the samples were then frozen at −70°C until the assay for free MHPG. All analyses were determined without knowledge of the source (arterial or venous) of the plasma samples. Quantitation was carried out by the mass spectrometric technique of selected ion monitoring, with the use of a model 3200 quadrupole mass spectrometer (Finnegan Corporation, Sunnyvale, California) equipped with a programmable multiple-ion monitor unit and electron impact ionization. To reduce the variance, plasma samples were assayed in duplicate and each of the duplicate samples was injected into the gas chromatograph-mass spectrometer (GC-MS) two or three times. The values from these multiple injections were then averaged to obtain a final value for a specimen. The values for duplicate plasma samples were averaged to obtain the final value for plasma MHPG. With this technique the coefficient of variation (standard deviation as a percentage of the mean value; $N = 10$) is 6.5 percent (*9*). Free MHPG was assayed, since almost all of the MHPG in human brain and *Macaca arctoides* brain is in the nonconjugated form (*10*). The *p*H and carbon dioxide (P_aCO_2) and oxygen P_aO_2) tensions, corrected to body temperature (Corning meter), were immediately measured, using 4 ml of the arterial samples (in heparinized syringes). Urine samples were obtained from six subjects, one patient and five normal volunteers, during a period of 1 to 3 1/2 hours, which bracketed the time during which the venous and arterial bloods were obtained. A urine specimen was obtained from one normal volunteer on a day after to the procedure but at the same time of day. Urine was assayed for total MHPG by GC-MS. Internal recoveries were calculated for individual samples and used to correct for losses that occurred during the hydrolysis. Unless otherwise noted, all values are reported as the mean ± standard error (S.E.).

Table 1 shows that in all cases the concentration of MHPG was higher in the internal jugular venous blood than in the arterial blood (paired *t*-test, one-tailed, $P < .0005$). The mean V-A difference for MHPG was 0.7 ± 0.1 ng per milliliter of blood. With this figure the average production of MHPG by human brain may be calculated as follows. The average human brain weighs 1400 g (*11*) and it is assumed that for our group of subjects the mean CBF is 60.9 ml per 100 g of brain per minute, a figure obtained by averaging reported values for total CBF from various studies [see table 1 in (*12*)]. It is therefore estimated that the rate of production of MHPG by whole brain is (0.7)(60.9)(14) ng/min = 597 ng/min or 35.8 μg/hour. The latter figure is in reasonable agreement with previous results for monkeys, which led to the estimate that the rate of production of MHPG by human brain would be 38 to 39 μg/hour (*6*).

It was found that for the six subjects (five normal and one patient) from whom urine was collected around the time blood samples were obtained, the quantity of MHPG excreted into urine averaged 64.5 ± 4.9 μg/hour. The mean V-A difference was 0.8 ± 0.2 ng for these six subjects. Using a standard brain weight and CBF as noted above, it is estimated that the mean rate of production of MHPG by whole brain in these six subjects was 40.9 μg/hour. The average contribution by brain to the total body production of MHPG is therefore estimated to be 63 percent.

As seen from Table 1, the range of the V-A difference in both patients and volunteers is quite large. It seems unlikely that this variation is a function of CBF; correlation of the V-A difference with P_aCO_2 was statistically nonsignificant, that is, the correlation coefficient $r = .06$. Furthermore, in previous studies with monkeys in which CBF was directly measured no correlation between CBF and the V-A value for MHPG or other neurotransmitter metabolites was found (*6*). Because all blood was obtained at approximately the same time of day, between 10:00 and 11:30 a.m., it also seems unlikely that diurnal variations accounted for the variance in the V-A values. It is, however, possible that because of anatomical variations in some cases a mixture of blood from the common facial vein and the internal jugular vein might have been obtained, thus diluting the venous concentration of MHPG. A relationship between the functioning of brain NE neurons and anxiety has been suggested (*13*) but has not been conclusively demonstrated. The variation in the V-A values may therefore be due to differences in the degree of anxiety produced by the procedure for obtaining the samples. However, only two of the normal subjects reported feelings of anxiety

of anxiety during the procedure and they had V-A differences of 1.2 and 0.4 ng/ml. The latter value is toward the lower end of the range (see Table 1) and is not consistent with a simple relationship between anxiety and the magnitude of the V-A difference.

Concerning the degree to which urinary MHPG reflects brain NE metabolism, the following points should be noted. In addition to MHPG, dihydroxyphenethyleneglycol (DHPG) is an important metabolite of brain NE (*4, 14*), and it is likely that a large fraction of this product on entering the body pool is *O*-methylated to form MHPG. Thus, the portion of the MHPG that is derived from DHPG, and therefore from brain NE metabolism, is not accounted for in the estimates given here. On the other hand, some of the MHPG formed in brain, on entering the body pool, may be converted to other products such as 4-hydroxy-3-methoxymandelic acid ["vanillylmandelic acid" (VMA)] and thus may not appear in urine as MHPG. For example, one group of investigators found that after an injection of deuterated MHPG into monkeys, 7 percent of the label was recovered as VMA; in contrast, another group found no labeled VMA in urine during a 24-hour period after the infusion of [^{3}H]MHPG into the internal jugular bulb of the monkey; and a third report indicates that in an infant with a neuroblastoma, 27 percent of an injected bolus of [^{3}H]MHPG was recovered as [^{3}H]VMA (*15*). Also, the mean P_aCO_2 for the subjects was 33.9 ± 1.1 torr, which is below the normal value of 40 torr, and because hypocapnia decreases CBF the actual CBF may have been less than that used to compute MHPG production per brain per minute.

In future work with humans, measurement of the V-A difference for a neurotransmitter metabolite coupled with a direct measure of CBF should yield information on brain neurotransmitter systems that will be of importance to investigators from a variety of disciplines.

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Pigeons Have Magnets

Abstract. *Research on pigeon homing suggests that magnetic field information is used for orientation. The ability of pigeons to sense magnetic fields may be associated with a small, unilateral structure between the brain and the skull which contains magnetite in what appears to be single domains.*

Many homing pigeons are able to find their way home after being released at an unfamiliar site. They typically take up an appropriate homeward flight direction shortly after release. To do this, the birds must know where they are with respect to home and be able to judge direction; that is, they must have both a "map" and a "compass sense" (*1*). On sunny days, their compass appears to depend on the sun since clock-shift experiments predictably deflect departure bearings (*2*). Since the same clock-shifted pigeons depart directly toward home on cloudy days, some nonsolar backup compass must be available (*3*). Because the ability of pigeons to orient on cloudy days is disrupted when small magnets (*4*) or paired coils (*5*) generating a magnetic field are affixed to their heads, it seems likely that the backup compass is partly or wholly magnetic, and is located in the head or neck. Since the initial homeward orientation of pigeons on sunny days is slightly affected by paired coils (*6*) and magnetic storms (*7*), and can be virtually abolished when the birds are released at strong magnetic anomalies (*8*), it is possible that the sunny-day compass has a magnetic component or that the mysterious "map" system may utilize magnetic field information.

Very little is known about how pigeons might sense the earth's field. However, we know of only three general strategies which an organism might use to detect magnetic direction. One method would be to measure the electric field (that is, charge separation) generated by moving a conductor through a magnetic field. This is almost certainly the system employed by elasmobranch fishes (*9*). Since the weak, static field of a set of coils attached to (and hence moving with) a pigeon nevertheless disrupts cloudy-day homing (*5*), the compass probably does not involve an induction detector. A second possibility would be to use permanent magnets, perhaps by measuring the torque generated as they attempt to twist into alignment with the earth's field (*10*). Chitons (*11*), honey bees (*12*), and many species of mud bacteria (*13*) do possess single domains (tiny unit magnets) of magnetite which, excepting chitons, may be used for magnetic field orientation. The third strategy might be to sense the effects of paramagnetic fields which are produced by

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