Central Norepinephrine Metabolism in Affective Illness: MHPG in the Cerebrospinal Fluid

Abstract. *Concentrations of the norepinephrine metabolite 3-methoxy-4-hydroxyphenyl glycol in cerebrospinal fluid were measured by a gas chromatographic method in 34 patients with affective illness and in 44 controls. Concentrations of this metabolite in spinal fluid were significantly lower in depressed patients than in controls or manic patients. These low values may occur secondary to depressive phenomena such as reduced psychomotor activity, or they may reflect a primary change in norepinephrine metabolism in depressive illness.*

The catecholamine hypothesis of affective illness, formulated on the basis of a variety of indirect evidence, suggests that functional concentrations of brain amines, particularly norepinephrine, are low in depression and elevated in mania (*1*). In an attempt to test this hypothesis, urinary excretion of the norepinephrine metabolite 3-methoxy-4-hydroxyphenyl glycol (MHPG) has been studied in several laboratories. Low urinary excretion of MHPG in depression and high excretion in mania have been reported (*2*). While a portion of urinary MHPG is derived from norepinephrine metabolism in the central nervous system (*3, 4*), the findings are clouded by the fact that peripheral sources do make a major contribution to urinary MHPG. Moreover, it was found in this laboratory that moderate increases in activity significantly increase 12-hour urinary excretion of MHPG in depressed patients (*5*).

In the present study, we attempted to more directly measure central norepinephrine metabolism through the use of cerebrospinal fluid (CSF) from patients with manic-depressive illness. Concentrations of MHPG, a principal metabolite of norepinephrine in the brains of various species (*3, 6*), were measured in human CSF with gas chromatographic methods (*7, 8*). We found that MHPG concentrations in the CSF of depressed patients are low compared to those in controls or manic patients (*9*). In contrast, Wilk *et al.* (*10*) have reported normal values for MHPG in the CSF of depressed patients and significantly higher values for manics.

Thirty-four patients with primary affective illness diagnosed by two psychiatrists and a social worker were studied on two metabolic research wards at the National Institute of Mental Health (NIMH). Only patients with depressive or manic illness severe enough to warrant hospitalization were admitted and studied. At the time of lumbar puncture, the clinical illness ranged from moderate to severe. Twice-daily ratings of depression, mania, anxiety, anger, and psychosis were made by nurse consensus on a 15-point scale. Half-hourly sleep checks were performed to estimate total sleep time. Patients were drug-free for 2 weeks before baseline lumbar puncture, but were in continuous individual and group psychotherapy throughout the hospitalization. Patients had been told of the ongoing lumbar puncture studies before voluntary admission to the hospital, and informed consent was obtained. Lumbar punctures were performed at 9 a.m. with the patient in the lateral decubitus position. Patients remained in bed and fasted from midnight until 9 a.m. Diets low in catecholamines and indoleamines were maintained. All medications, including sedatives, salicylates, and alcohol, were rigorously avoided.

The control populations consisted of ten normal parents of children admitted for neurological evaluation and 34 neurological patients with a variety of central nervous system illnesses studied under similar baseline conditions at the NIMH in collaboration with T. N. Chase (*11*). Determinations of MHPG were performed on a sample from the first 8 ml of CSF removed. The gas-liquid chromatography method of Gordon and Oliver (*8*) was employed.

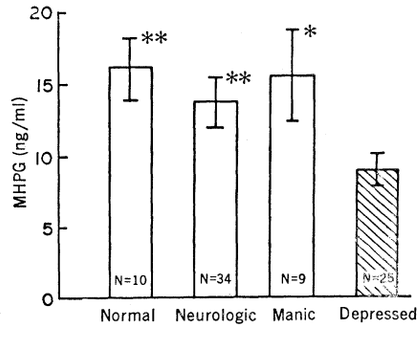

Fig. 1. Low MHPG concentration in cerebrospinal fluid of depressed patients. Levels of MHPG in depressed patients were significantly lower than in other groups (*, $P < .05$; **, $P < .01$; Student's *t*-test, two-tailed). Normals and neurological patients were studied in collaboration with T. N. Chase.

Depressed patients had significantly lower MHPG concentrations in CSF than did controls ($P < .01$) or neurological ($P < .01$) or manic patients ($P < .05$) (Fig. 1). Values for MHPG in depressed patients (8.9 ± 1.1 ng/ml; mean ± standard error of mean) were also lower than in a subgroup of 14 patients with Parkinsonism (14.8 ± 2.4 ng/ml) ($P < .05$). Concentrations of MHPG in depressed patients were not related to the presence or absence of a history of mania. Patients who subsequently responded to tricyclic antidepressant medications had MHPG values similar to nonresponders. Degrees of rated depression, agitation, retardation, psychosis, anxiety, and anger were not significantly correlated with MHPG values. Age, weight, and hours of sleep on the night before lumbar puncture were also not significantly related to MHPG concentrations.

In manic patients, there was no correlation of the degree of mania with MHPG values. However, the manic patient with the lowest MHPG concentration (5 ng/ml) was atypical in that he displayed no motor hyperactivity and had no difficulty with bed rest before lumbar puncture.

Gordon *et al.* (*12*) found small but significant increases in free MHPG, but not in the sulfate conjugate, after subjects were treated with probenecid (100 mg per kilogram of body weight) for 18 hours. The probenecid-induced increases that we found for total MHPG were consistent with the observed baseline MHPG differences. The MHPG levels after 18 hours of probenecid treatment (100 mg/kg) were lower in 21 depressed patients (14.3 ± 1.9 ng/ml) compared to five neurological controls (22.4 ± 3.0 ng/ml) ($P < .05$; Student's *t*-test, two-tailed); the same trend was seen in comparison to eight manic patients (21.0 ± 4.7 ng/ml).

Our results differ from those of Wilk *et al.* (*10*), who found normal MHPG concentrations in depression and higher values in some manic patients. In their study, lumbar punctures were performed later in the morning, after patients had been active. Bed rest was maintained for only half an hour. In contrast, our patients fasted and remained at bed rest for 9 hours before the 9 a.m. lumbar puncture. This difference in prior activity could be critical, because we showed that 4 hours of activity prior to lumbar puncture raised MHPG levels in CSF of depressed

patients into the normal range (*13*).

While evidence from studies in animals (*3, 6*) suggests that MHPG is a major metabolite of brain norepinephrine, the relation of amine concentration in the brain to metabolite concentration in the CSF is not clear. Significant amounts of labeled MHPG administered intravenously in three patients did not appear to enter the CSF (*14*). Therefore, a source of MHPG in the central nervous system is likely. There is no rostral-caudal CSF gradient for MHPG (*8*), and a probenecid-sensitive transport mechanism does not appear to be a major mechanism for elimination of MHPG (*12*), as is the case for the acid metabolites of dopamine and serotonin. In animals, norepinephrine is abundant in the spinal cord and appears localized to nerve endings of descending tracts, which degenerate after spinal cord transection (*15*). Our study of 13 patients with spinal cord transection, with and without spinal fluid block, suggests that MHPG concentrations in CSF reflect norepinephrine metabolism in spinal cord as well as in brain (*16*). Patients with transected spinal cords had lower MHPG values than did controls, and results were similar in the presence and absence of block in CSF flow.

Although part of the MHPG measured in lumbar CSF may be derived from spinal cord sources, the results reported here suggest that norepinephrine metabolism in the central nervous system may be reduced in depression. The low MHPG values in the CSF of depressed patients support and extend the reports of low MHPG excretion in the urine of depressed patients (*2*).

However, the implication that our data support a primary role of catecholamines in affective illness should be viewed critically. Activity appears to increase MHPG concentration in CSF (*13*), and low levels of MHPG have been found in the CSF of para- and quadraplegics where limited activity may also be a factor (*16*). Thus, the low MHPG concentrations in the CSF of depressed patients may occur secondary to the behavioral inhibition associated with the depression rather than reflect a primary role for brain norepinephrine in the pathogenesis of affective illness. However, the lack of correlation of MHPG with agitation-retardation within the depressed or manic groups appears to indicate that whereas activity may be an important determinant of amine metabolite concentrations in CSF, it clearly is not the only one. In addition, MHPG values in CSF of depressed patients were significantly lower than those in Parkinsonian patients, who also manifest a considerable degree of motor inhibition.

Our results lend support to the idea that central norepinephrine metabolism is altered in depression. Whether this change is etiologically related to depressive illness or is secondarily related to its clinical and biological concomitants remains to be determined.

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Hormonal Regulation of Growth in Unfertilized Cotton Ovules

Abstract. *Exogenous plant growth regulators can substitute for pollination, fertilization, and subsequent embryo development in cotton. Isolated, unfertilized, immature ovules enlarge in the presence of kinetin, and both enlarge and produce fibers in the presence of indoleacetic acid or gibberellic acid or both. An extract of germinating cotton pollen qualitatively mimics the effect of exogenous hormones.*

Much information concerning the physiology of fruit development and embryogenesis could be obtained if the component organs involved in sexual reproduction could be cultured separately in vitro. Recent literature has described the culture procedures required and hormonal interactions involved in the in vitro development of fertilized cotton ovules and their associated fiber cells. In brief review, the cotton fiber is a single epidermal cell that begins to elongate from the ovule surface at anthesis. If fertilization is accomplished the ovule may be successfully cultured in vitro (*1*), and both fibers (*2*) and embryos (*3*) are produced. This report describes the next basic step, that of the successful in vitro culture of immature cotton seeds, without zygote.

In angiosperms, fusion of the sperm nucleus and the egg nucleus creates the zygote and, for most species, fusion of another sperm nucleus with two polar nuclei of the embryo sac triggers formation of the nutritive endosperm. These creative unions initiate a myriad of biochemical reactions ultimately producing a mature fruit (ovary), containing its seeds (ovules), which in turn house the new plants (embryos).

Examples of the successful culture of excised plant embryos are available from the major groups of seed plants, for example, a gymnosperm (*4*), a dicotyledonous angiosperm (*5*), and a grass (*6*). Isolated plant ovaries, containing fertilized ovules, have been cultured in vitro, giving rise to enlarged fruits and germinable seed (*7, 8*). Seedless fruits from unpollinated flowers have been obtained in vitro by the incorporation into the medium of auxin-