Selective Antidepressants and Cerebrospinal Fluid

Lack of Specificity on Norepinephrine and Serotonin Metabolites

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• Cerebrospinal fluid concentrations of the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and the dopamine metabolite, homovanillic acid, were measured in depressed patients before and after treatment with three putatively specific antidepressants. The expected specificity of action on these three neurotransmitter metabolites was not observed. Desipramine hydrochloride, a norepinephrine uptake inhibitor, reduced 5-HIAA as well as MHPG concentrations; zimeldine hydrochloride, a serotonin uptake inhibitor, reduced MHPG as well as 5-HIAA concentrations; and clorgyline, a selective monoamine oxidase type A inhibitor, which might be predicted to most affect 5-HIAA, dramatically reduced MHPG, moderately reduced homovanillic acid, and only modestly reduced 5-HIAA concentrations.

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The interpretation of numerous clinical investigations has been based on the presumed specificity of action of various antidepressants as inhibitors of either norepinephrine or serotonin (5-HT) reuptake.[1-9] These have been based on the classic preclinical characterizations of tertiary amine tricyclic antidepressants as serotonin uptake inhibitors and of secondary amine tricyclic antidepressants as norepinephrine uptake inhibitors.[10] Recent animal studies have focused on the ability of antidepressants administered long term, no matter what their initial biochemical effect, to alter noradrenergic and serotonergic receptor number and/or function.[11,12] The underlying logic of the latter approach stems from the generally accepted clinical observation that true antidepressant response in man requires ten to 21 days no matter what the treatment. Clinical pharmacologic studies of drug action have focused, therefore, on drug effects observed after at least a week of treatment.

Briefly, all such investigations, whether looking for antidepressant treatment-associated biochemical changes in urine, blood, or cerebrospinal fluid (CSF), have revealed reductions in a major metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG).[13] A few studies have provided indirect evidence of predominant effects on the serotonergic system after certain antidepressants; blockade of 5-HT uptake by platelets isolated from patients before and after treatment has been the usual measure.[14,15] Direct measurement of the major 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the CSF before and after treatment has also been used to show effects of certain antidepressants, including monoamine oxidase inhibitors, on serotonin.[2,16-23] In general, the results have been interpreted to support a relative specificity of action on a single neurotransmitter system.

Based on these conventional assumptions, we investigated the three antidepressants clinically available to us with the greatest known specificity of action: a norepinephrine uptake inhibitor, desipramine hydrochloride, a nontricyclic serotonin uptake inhibitor, zimeldine hydrochloride, and a monoamine oxidase type A inhibitor, clorgyline. We used urinary output of norepinephrine and its major metabolites before and after treatment to assess drug effects on "whole body" norepinephrine turnover and metabolism. As previously reported, we found that all three treatments reduced total turnover while exerting differential effects on the various metabolic pathways of norepinephrine, the latter findings being consistent with preclinical data on each drug's effects at the synapse.[24] The reduction of norepinephrine turnover by zimeldine was something of a surprise. In preliminary trials, we and others had not observed a clear decrease of MHPG in the CSF of patients treated with zimeldine.[6] We report herein subsequent and more extended analyses of CSF changes that show that all three treatments not only produce common effects on the norepinephrine system as reflected in CSF MHPG but also on the 5-HT system as reflected in 5-HIAA.

PATIENTS AND METHODS

Sex, age, diagnoses, and treatments for the 25 patients participating in the study are given in Table 1. The diagnoses made according to the *Research Diagnostic Criteria*[25] represent the consensus of two psychiatrists who independently interviewed and observed the patients after at least a two-week period of hospitalization on our research unit. Before inclusion in the study, patients had received no psychotropic medications for at least three weeks. During the study they received a low-monoamine diet.[26]

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Table 1.—Patient Characteristics

Patient/ Age, yr/Sex	Diagnosis*	Treatment		
		Desipramine Hydrochloride	Zimeldine Hydrochloride	Clorgyline
A/69/F	UP	X	X	...
B/29/F	BP I	...	...	X
C/54/F	UP	...	...	X
D/53/F	UP	...	X	...
E/29/F	UP	X	...	...
F/55/F	BP I	...	...	X
G/34/F	BP II	X	X	...
H/58/M	UP	X	...	...
I/53/F	BP II	X	...	...
J/28/F	BP I	X	X	...
K/70/F	UP	...	...	X
L/22/F	UP	X	...	...
M/32/M	UP	X	...	...
N/21/M	BP I	...	...	X
O/53/F	BP I	...	...	X
P/42/F	UP	X	...	...
Q/57/F	BP I	...	...	X
R/48/F	UP	X	X	...
S/65/M	UP	X	...	...
T/61/F	BP II	...	X	X
U/18/F	BP II	...	X	...
V/35/F	BP II	...	...	X
W/42/F	UP	...	...	X
X/24/F	UP	...	X	...
Y/28/F	UP	...	X	...

*UP indicates unipolar depression; BP_I, bipolar depression type I; BP_{II}, bipolar depression type II.

Table 2.—Drug Effects on MHPG, 5-HIAA, and HVA*

	Treatment Group		
	Desipramine Hydrochloride (N=11)	Zimeldine Hydrochloride (N=9)	Clorgyline (N=10)
Pretreatment MHPG	46±3	46±4	47±3
Posttreatment MHPG	28±2	37±2	13±2
Δ MHPG	−18±2	−9±3	−34±4
Δ MHPG, %	−40	−20	−72
Pretreatment 5-HIAA	117±13	102±9	110±13
Posttreatment 5-HIAA	79±7	62±8	82±8
Δ MHPG	−38±10	−40±5	−28±8
Δ MHPG, %	−32	−39	−26
Pretreatment HVA	212±35	158±10	194±30
Posttreatment HVA	173±24	171±22	132±20
Δ HVA	−40±19	+13±17	−16±12
Δ HVA, %	−19	+8	−32

*MHPG indicates 3-methoxy-4-hydroxyphenylglycol; 5-HIAA, 5-hydroxyindoleacetic acid; and HVA, homovanillic acid.

The treatment of 12 of the patients was as previously described[27] following a crossover design of zimeldine to desipramine or vice versa with a three- to four-week interdrug placebo period and four- to six-week period receiving drug. Not all patients completed the crossover treatment, and it was only possible to obtain the series of three lumbar punctures (see below) on five of the patients. One of the patients who was part of the zimeldine/desipramine crossover was ultimately treated with clorgyline. She and two other of the ten patients treated with clorgyline were the subject of a previous clinical report on the efficacy of clorgyline.[28] A preliminary report of the clinical response of the additional patients treated with clorgyline has been presented.[29] Biochemical studies on urinary measures of dopamine and 5-HT before and after treatment with clorgyline in seven of these patients have also been reported.[30,31] To our knowledge, six of the 25 patients have not been the subject of any previous report.

Lumbar punctures were performed during a baseline placebo period when the mean depression score for a week was greater than 5 on the twice-daily Bunney-Hamburg Scale[32] with nurse raters "blind" to medication and patients free of psychotropic medications for at least three weeks. The procedure was as follows: Lumbar CSF was collected in the lateral decubitus position between 9 and 10 AM after at least eight hours of bed rest. The first 12 mL of CSF was mixed thoroughly, aliquoted in 1-mL portions, frozen immediately, and stored at −60 °C without preservatives or with 0.05% to 0.1% ascorbic acid.

Between three and four weeks of receiving desipramine or zimeldine, a repeated lumbar puncture was performed under identical conditions. Following treatment with clorgyline, repeated lumbar punctures were performed from five to eight weeks after beginning clorgyline treatment. Concentrations of the following substances were determined in CSF under each condition: MHPG, 5-HIAA, and homovanillic acid (HVA) using high-performance liquid chromatography (HPLC) with electrochemical detection.

The method of Scheinin et al[33] was used to quantify the metabolites. In brief, after gently thawing a 1-mL sample of CSF, aliquots (200 to 500 μL) were mixed with 20 to 50 μL of a 5-μM solution of 5-fluoro-homovanillic acid as an internal standard. The resulting solution was filtered through a disposable membrane using a micropartition system (Amicon MPS-1). A 100-μL sample of the filtrate was then injected onto the HPLC column. A series of aqueous standard solutions of MHPG, 5-HIAA, and HVA were similarly prepared. A mobile phase of 0.1M sodium acetate with 0.01M citric acid and 0.25mM disodium ethylenedinitrotetraacetate and methyl alcohol (92:8) was used (final pH 5.22±0.02). Instrumentation consisted of a pump (Altex 110) equipped with a pulse dampener, an injector (Altex 210), a self-packed precolumn kit (Waters) (C-18), an analytical column (Ultrasphere ODS) (250×4.6 mm, 5-μm particle size), an electrochemical detector (LC4A) equipped with a glassy carbon electrode in a flow cell (TL-5), and a dual-channel recorder. Flow rate was 1.3 mL/min and detection was at a flow cell potential of 0.75 V *v* silver/silver chloride, wih the sensitivity at 5 or 10 nA. Norepinephrine in CSF was also analyzed using HPLC with electrochemical detection.[34]

Venous blood samples were drawn just prior to each lumbar puncture. Those obtained while the patient was receiving desipramine or zimeldine were analyzed for plasma concentrations of parent drug and active metabolites, as previously described.[35]

Pretreatment and posttreatment metabolite levels were compared using *t* tests for related samples. The changes in metabolite levels among the three different treatments were analyzed with *t* tests based on equal or unequal variances, as appropriate. Two-tailed probabilities were used in all comparisons. Fractional change in CSF monoamine metabolite levels was correlated with the plasma drug concentration, after logarithmic transformation of the latter, using Pearson's product-moment correlation coefficient. This test was also used in correlating CSF 5-HIAA and HVA concentrations. The change in the correlation of CSF 5-HIAA with HVA following drug treatment was analyzed with Fisher's *z* transformation.[36,37]

RESULTS

Pretreatment concentrations of MHPG, 5-HIAA, and HVA in CSF did not differ among the three groups of patients who received desipramine, zimeldine, or clorgyline (Table 2). Mean values of both MHPG and 5-HIAA were reduced after all three treatments, but the HVA value decreased only after clorgyline (Table 2). The extent and pattern of change did tend to differ among treatments.

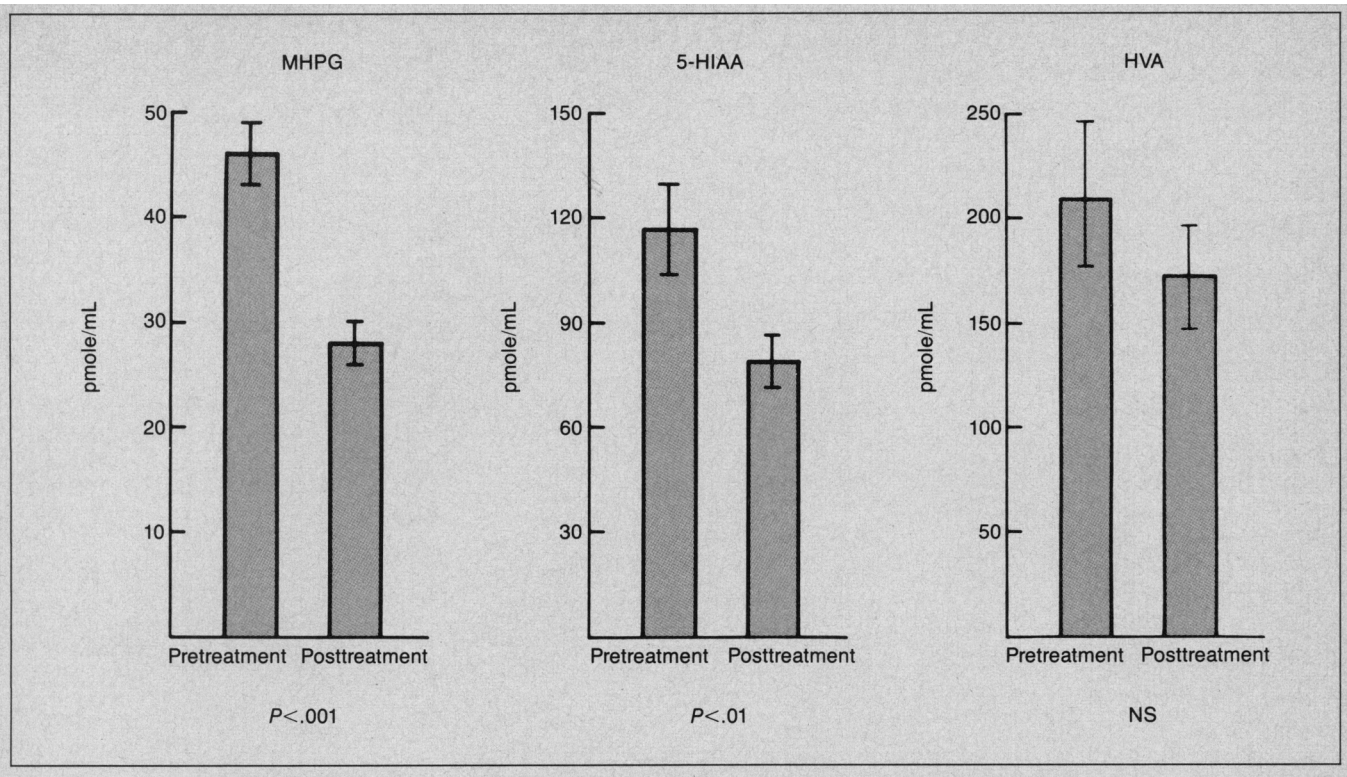

Fig 1.—Effects of long-term treatment with desipramine hydrochloride on cerebrospinal fluid neurotransmitter metabolites. MHPG indicates 3-methoxy-4-hydroxyphenylglycol; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; NS, not significant. Bars show mean values ± SEM in 11 patients before and after treatment.

Desipramine

All subjects showed moderate reductions of CSF MHPG concentration (Fig 1). The MHPG reduction was significantly less than that observed after clorgyline ($P<.005$). Reductions of 5-HIAA concentrations were more variable. The fractional decrease of CSF 5-HIAA concentration (32%) was not significantly different from that observed after zimeldine (39%) or clorgyline (26%). The correlation of .93 between 5-HIAA and HVA observed prior to treatment was reduced to .55 ($P<.002$) by desipramine.

Zimeldine

The MHPG concentration decreased in all but one patient (Fig 2); the decrease was significantly less than that observed after clorgyline ($P<.001$). The 5-HIAA concentration decreased in all patients by more similar absolute amounts; the overall fractional decrease was significantly greater than that observed with clorgyline ($P<0.05$) but not desipramine. The correlation coefficient between 5-HIAA and HVA was unchanged after zimeldine (.75 *v* .85), although the slope of the regression line increased from .85 to 2.28.

Clorgyline

The MHPG concentration decreased by 72%, HVA by 32%, and 5-HIAA by 26% (Table 2 and Fig 3). The fractional decrease of the HVA concentration after clorgyline treatment was greater than the trend toward a decrease observed after treatment with desipramine and dramatically different from the trend toward an increase after zimeldine ($P<.003$). In contrast, the fractional decrease of 5-HIAA concentration, although highly significant, was no different from that observed after treatment with desipramine and was significantly smaller than after zimeldine.

Relationships between steady-state plasma concentrations of drug and CSF concentrations of MHPG, 5-HIAA, and/or HVA were examined by comparing the fractional change associated with drug treatment *v* the $\log_{10}$ drug concentration. There was a borderline significant relationship between the concentration of desipramine and reduction of 5-HIAA ($r=-.55$, $P<.05$, $n=11$) but not of MHPG over the observed range of plasma concentrations (75 to 300 ng/mL). Concentrations of zimeldine and/or norzimeldine, its active metabolite, in the ranges observed, were not significantly related to changes in neurotransmitter metabolites.

COMMENT

These results demonstrate that previous assumptions based on preclinical studies concerning the effects of biochemically "specific" antidepressants are incorrect: When these studies were designed six years ago, we and others assumed that selective inhibitors of norepinephrine or 5-HT uptake would produce selective alterations in the respective system and be specific for biochemical subtypes of depression.[2-7,10] Many other types of data have appeared during recent years that at least indirectly challenge such an assumption of specificity.[38] For example, some investigators report 90% response rates to norepinephrine uptake inhibitors (desipramine and nortriptyline) when dose and/or plasma concentrations are adjusted[39,40]—this would argue against a subpopulation needing a more "serotonergic" antidepressant. Against expectation, the clinical efficacy of clomipramine hydrochloride was more related to the presence of its potent norepinephrine uptake–inhibiting metabolite than to the ability of parent drug to inhibit 5-HT uptake.[8] Preclinical studies consistently show that all antidepressant treatments regardless of in vitro properties reduce noradrenergic β-receptor number or function when administered long-term to rats.[12,41,42] It is of note that most also reduce the number of serotonergic receptors and that interactions between the noradrenergic and serotonergic

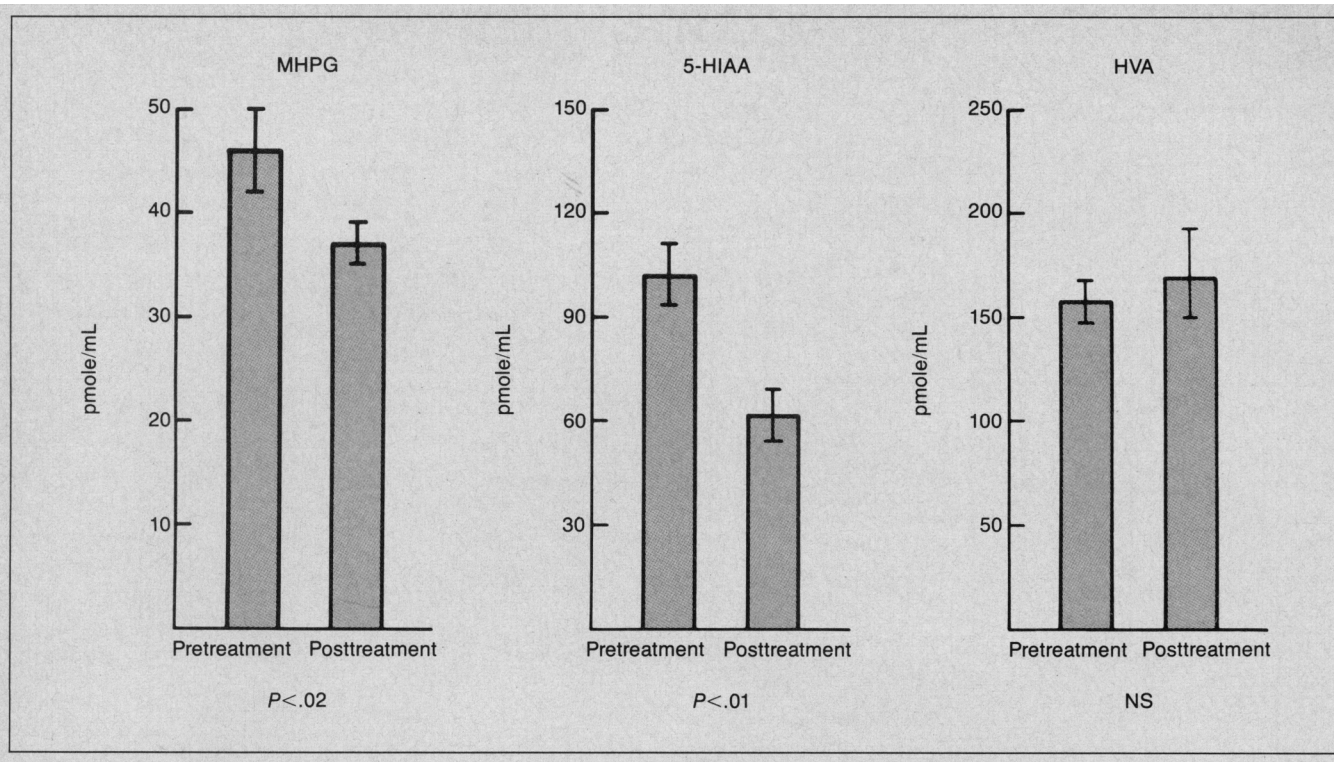

Fig 2.—Effects of long-term treatment with zimeldine hydrochloride on cerebrospinal fluid neurotransmitter metabolites. Abbreviations as in Fig 1. Bars show mean values ± SEM in nine patients before and after treatment.

systems occur (see below). Nonetheless, investigators still tend to interpret data such as a statistical relationship between pretreatment concentration of 5-HIAA in CSF[1,43] or of MHPG in urine[7,44] and outcome on 5-HT and norepinephrine uptake inhibitors as indicating specificity of drug action. Low MHPG concentration, the most consistently reported predictor of response, however, is now reported to work as well with amitriptyline, a mixed uptake inhibitor, as with more putatively selective compounds.[44]

It also appears that "selective" uptake inhibitors produce reductions of both 5-HT and norepinephrine as measured by CSF concentrations of their major metabolites, 5-HIAA and MHPG. Bertilsson et al[45] have already noted that contrary to initial impressions,[22,27] zimeldine reduced MHPG concentrations in CSF by 11%. We now find that with improved clinical (ie, longer drug-free pretreatment period) and analytical techniques, zimeldine produces a highly significant 20% reduction in CSF MHPG concentrations. This is consistent with our findings of reduced urinary MHPG output after zimeldine therapy in patients[24] and volunteers.[46] As far as we know, ours is the first demonstration that an uptake inhibitor as selective as desipramine for norepinephrine in moderate doses can consistently reduce 5-HIAA concentrations in the CSF. It has been reported by the Karolinska group that nortriptyline reduces 5-HIAA concentrations in the CSF,[17] but it is known to be twice as potent as desipramine in inhibiting 5-HT uptake.[47,48] Moreover, the five subjects studied who received both drugs showed changes characteristic of the group; direction and fractional extent of biochemical change were a function of drug and not of the individual.

There are at least two explanations for the current findings with desipramine and zimeldine. The first and simplest is that there is enough drug in the system to produce mixed uptake inhibition, no matter what the in vitro specificity. We cannot rule out this possibility in the case of desipramine reducing the 5-HIAA concentration. Since higher concentrations of desipramine tended to be associated with greater reductions of 5-HIAA values and since it has previously been shown that higher concentrations of desipramine (>150 ng/mL) reduce 5-HT uptake by platelets from patients by over 40%,[49] it is possible desipramine's effects on 5-HIAA can be accounted for on the basis of its 5-HT uptake inhibition. In our patient population, only four of 11 patients had desipramine concentrations above 150 ng/mL. Moreover, since the known 5-HT uptake inhibitor zimeldine produced a different pattern of 5-HIAA change than did desipramine, at least in terms of the relationship of 5-HIAA to HVA, other mechanisms of desipramine's effects need to be considered. It may be possible that primary noradrenergic actions (eg, such as norepinephrine uptake inhibition after desipramine) can produce secondary serotonergic alterations. Indeed, several preclinical studies have shown that, in order for antidepressants to affect noradrenergic receptors, the serotonergic system must be intact[50,51] and that serotonergic functions are modified by noradrenergic input.[52-54] Since desipramine increases noradrenergic function in man, even after long-term treatment,[55] we consider the possibility that much of the effect on 5-HIAA is secondary to interactions between the norepinephrine and 5-HT systems rather than any nonspecificity of the drug in terms of 5-HT uptake inhibition.

In contrast, we found no evidence that the effect of zimeldine on MHPG values was related to drug concentration. Moreover, in previous studies we showed that despite overall reductions, the relative urinary excretion of norepinephrine and its metabolites—normetanephrine, vanillyl-

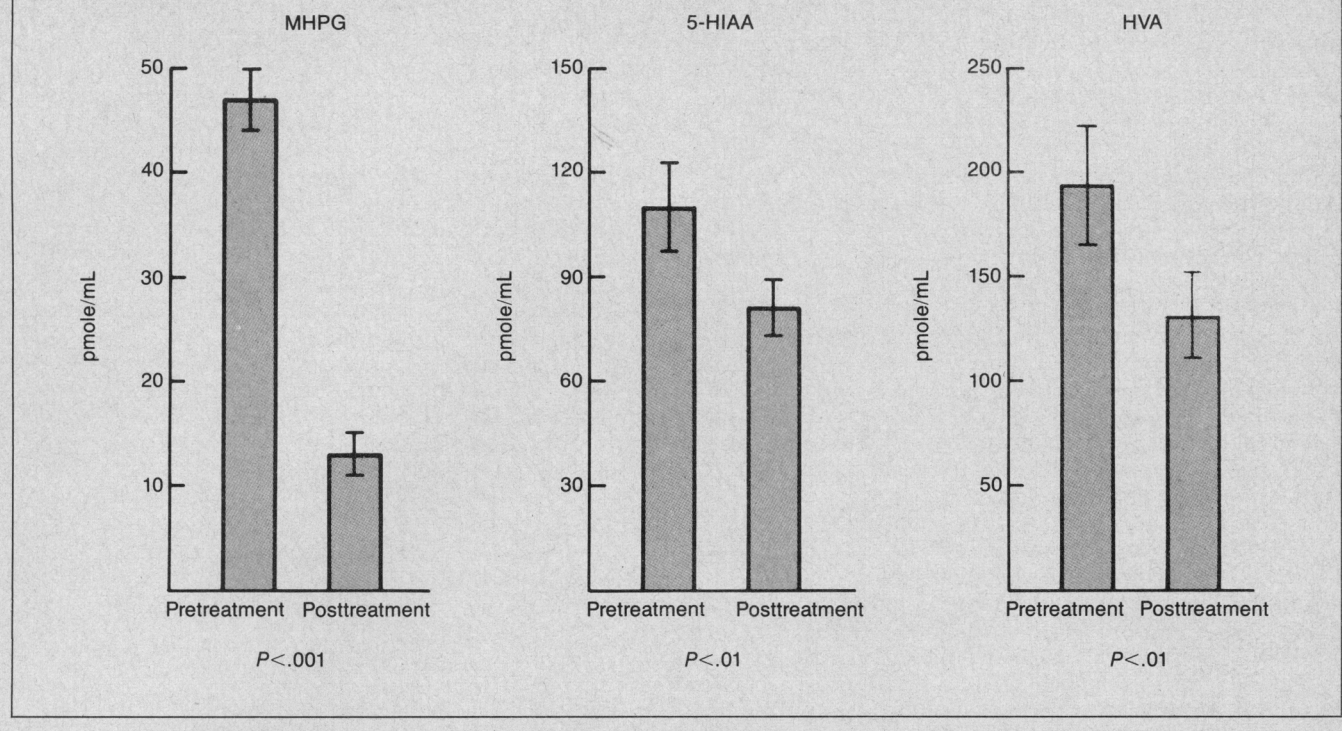

Fig 3.—Effects of long-term treatment with clorgyline on cerebrospinal fluid neurotransmitter metabolites. Abbreviations as in Fig 1. Bars show mean values ± SEM in ten patients before and after treatment.

mandelic acid, and MHPG—did not change after administration of zimeldine to these same patients[24] or to volunteers.[46] Since norepinephrine uptake inhibition shifts the relative urinary excretion in favor of normetanephrine,[24] we believe that the effects of zimeldine are most likely due to an overall reduction in the formation and/or release of norepinephrine itself and not due to norepinephrine reuptake inhibition. This interpretation is consistent with animal studies showing that 5-HT uptake inhibitors can reduce the turnover of norepinephrine in the brain as well as down-regulate noradrenergic β-receptors.[56-58]

Clorgyline and Monoamine Oxidase (MAO)–Type A

Before considering the three treatments together, we will consider our mistaken assumption concerning MAO-A inhibition. In vitro studies led to the expectation that the preferred substrate for A inhibition, and hence the one showing the greatest effect after clorgyline, was 5-HT. After all, it is by using 5-HT as a substrate that clorgyline is classified as an MAO–type A inhibitor.[59,60] Murphy et al[61] have previously noted that in a group of patients treated with 30 mg/day of clorgyline, the 5-HIAA decrease was not as pronounced as expected or as great as that of MHPG (45% *v* 91%). In our patients treated with 5 to 10 mg/day of clorgyline, this discrepancy is more marked as a proportion; ie, 5-HIAA decreased by only 26% *v* 72% for MHPG and 32% for HVA. Moreover, the 5-HIAA decrease after clorgyline was the smallest observed after any of the current treatments. The HVA data are also important since dopamine is characterized in vitro as a less preferred substrate of MAO-A than serotonin but in these patients HVA data show, if anything, a greater fractional reduction than 5-HIAA (32% *v* 26%). Finally, it is now clear that even in the rat, in order to obtain in vivo inhibition of 5-HT deamination, one must combine both MAO-A (eg, clorgyline) and MAO-B (eg, deprenyl) inhibitors.[62-64]

The in vivo action of clorgyline has therefore emerged as quite different than originally conceptualized and clearly favors inhibition of deamination in the following order: norepinephrine is greater than dopamine which is greater than 5-HT. The reason for this is unclear but may involve selective concentration of clorgyline into the noradrenergic and dopaminergic neurons or differential presence of MAO-A and MAO-B activities within the different monoaminergic neurons.

Common *v* Unique Actions

The present findings of reduction of MHPG concentrations in the CSF after all three treatments are both qualitatively and quantitatively similar to the drug effects on urinary MHPG. Since most of the subjects were the same in the current investigation of CSF as in the previous ones on urine, it appears that MHPG changes in either compartment may provide the same information. Despite finding rather weak correlations between MHPG in urine and CSF in earlier studies, we currently observe a relatively strong relationship ($r = .7$), at least with depressed inpatients receiving a controlled diet who have been free of medication at least three weeks and from whom at least three 24-hour urine samples are available close to the time at which the CSF is obtained. Presumably, plasma MHPG would show the same pattern of decrease and has in subjects treated with desipramine[65,66] or clorgyline.[67] Thus, under controlled conditions, MHPG would appear to be in equilibrium in the three available compartments, CSF, blood, and urine, and be reduced by three specific antidepressants.

This common MHPG reduction evidently reflects a reduc-

tion of norepinephrine synthesis and release since the sum of norepinephrine plus its other metabolites is reduced in urine after each of the treatments.[24,28] As noted above, differences in degree of reduction are consistent with altered routes of norepinephrine metabolism after clorgyline and desipramine but not after zimeldine.

The CSF may provide unique information on the 5-HT metabolite 5-HIAA—at least findings are much clearer than in urine.[31] Since all three treatments significantly reduced 5-HIAA concentration and since antidepressant-induced noradrenergic β-receptor changes in rodent brain can be blocked by lesions of the 5-HT system,[50,51] the current study is compatible with a 5-HT involvement in the action of antidepressants. Again, the only specificity observed was in terms of magnitude, not direction, of change; 5-HT uptake inhibition after zimeldine might be expected to produce a shift away from 5-HIAA, although other metabolic products of 5-HT in the CSF were not documented in these subjects. In any event, as described above, preclinical studies now show clear interactions between the noradrenergic and serotonergic systems, suggesting that it may be impossible to alter one system without altering the other, at least after a week or two of treatment.

Such considerations also call into question inferences on a serotonergic *v* noradrenergic etiology of depression based on apparent preferential response to one type of antidepressant or another. Most such reports have involved small numbers of subjects and the results could have occurred by chance, a likely explanation in light of many studies showing high response rates in nondelusional endogenous depression to a single agent if dose, blood level, and/or duration of treatment are controlled.[39,40] Interestingly, excluding electroconvulsive therapy, clorgyline with its pronounced effect on the noradrenergic system has been the most effective antidepressant in the seriously ill population referred to us.[29]

Murphy et al,[61] who treated patients with a much higher average dose of clorgyline, noted that clorgyline was particularly effective in patients who exhibited endogenous symptoms and had pretreatment Hamilton Depression Scale scores over 30. Furthermore, antidepressant effect was most closely related to changes in the noradrenergic system.[68]

We turn now to the one factor that qualitatively distinguished our drug treatments, HVA. The reduction of HVA concentrations after clorgyline could result simply from an expected partial inhibition of deamination since dopamine is a substrate for both MAO type A and B.[63] Our data on the urinary excretion of dopamine and its metabolites suggest, however, that after clorgyline the total production and release of dopamine is reduced as well.[30] The variable changes of HVA in the CSF after desipramine and zimeldine are consistent with the lack of significant effect of these drugs on the urinary excretion of dopamine and its metabolites.[30] These data indicate that antidepressants do not necessarily affect the dopaminergic system. Moreover, they argue against the suggestion that effects of 5-HT and norepinephrine uptake inhibitors may be mediated by overlapping inhibition of dopamine uptake.[69] On the other hand, the dopaminergic system may prove to have particular relevance for bipolar illness as suggested by our earlier finding that lithium carbonate and clorgyline, two drugs known to affect cycle length, reduce dopamine turnover in man.[30]

In conclusion, we would emphasize that our findings on biochemical effects of antidepressants in man again demonstrate the necessity of testing assumptions based on preclinical models before drawing broad theoretical inferences. Furthermore, advances in methodology, both in terms of controlling sources of variance and assaying samples, have caused us to question ours and others' assumptions concerning the ability of CSF, plasma, or urine to give unique information about a neurotransmitter. Perhaps we will find that one body fluid is preferable from a practical point of view to characterize a person's biochemical state; if equilibrium is achieved, however, there is no known reason why they should not yield equivalent information, at least in terms of MHPG. Looking to the future, we believe that studies designed to further understand the working of the norepinephrine system in man will yield information critical to elucidating the mechanism of action of antidepressant drugs and perhaps the etiology of affective illness itself. Given our data in man and the cited preclinical data, a necessary corollary of studying the norepinephrine system is a continued examination of the serotonergic one.

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