Antidepressants Reduce Whole-Body Norepinephrine Turnover While Enhancing 6-Hydroxymelatonin Output

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• **The effects of antidepressant treatment on noradrenergic function were studied in 27 patients with a major affective disorder. Twenty-four–hour urinary excretion of 6-hydroxymelatonin and "whole-body norepinephrine (NE) turnover," ie, 24-hour urinary output of NE and its major metabolites 3-methoxy-4-hydroxyphenylglycol, vanillylmandelic acid, and normetanephrine, were measured before and after treatment with the tricyclic desipramine hydrochloride, the aminoketone bupropion hydrochloride, the nonselective monoamine oxidase (MAO) inhibitor tranylcypromine sulfate, and the specific MAO type A inhibitor clorgiline. 6-Hydroxymelatonin excretion increased following antidepressant treatment, while at the same time whole-body NE turnover was reduced. These findings support the hypothesis that antidepressant therapy increases noradrenergic "efficiency," in that functional output, as measured by 6-hydroxymelatonin, is maintained while total NE production is decreased.**

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For more than two decades, noradrenergic systems have been a central focus in theories relating to the pathophysiology of depression and the mechanism of action of antidepressants. The original formulations[1-3] of the biogenic amine hypothesis of depression were advanced at a time in which emerging technology permitted the measurement of a major norepinephrine (NE) metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), in body fluids. Also, the first-generation antidepressant medications shared the common property of having acute biochemical effects on NE: monoamine oxidase (MAO) inhibitors affect the enzyme responsible for the intraneuronal degradation of NE; secondary amine tricyclic antidepressants block reuptake of NE following its extraneuronal release; tertiary amine tricyclic antidepressants are metabolized to secondary amines and consequently also affect NE reuptake.[4,5]

More recent observations have called into question the central role of NE in antidepressant treatments. Newer agents that do not have acute biochemical effects on NE have been used to treat depression (eg, specific serotonin reuptake inhibitors such as citalopram and zimelidine).[6] With the recognition that down regulation of noradrenergic receptors following antidepressant administration coincides temporally with the time course of clinical response, some researchers have suggested that decreased noradrenergic functioning may be an essential factor in antidepressant effects.[7-9]

There is a growing appreciation for the dynamic nature of neurotransmitter systems. Thus, it becomes difficult to interpret the physiologic meaning of a static measurement of a single component of the system in any given compartment. For example, if low levels of MHPG were to be found in the cerebrospinal fluid of a group of patients, would this represent a primary event (eg, decreased output of NE and a resultant decrease in noradrenergic function) or a secondary compensatory event (eg, response to increased receptor sensitivity and/or density in a hyperactive system)? Limitations in interpreting monoamine metabolite tissue concentrations have stimulated a search for meaningful physiologic indexes of noradrenergic functioning.

Melatonin formation may prove to be a useful tool in this regard. As reviewed by Lewy,[10] several factors suggest that pineal gland secretion of melatonin could be utilized as an index of noradrenergic tone in man. The pineal gland is unique in that, unlike other endocrine organs, it is regulated primarily by neural (sympathetic) innervation and does not appear to be affected by circulating substances. Parasympathetic innervation does not seem to be

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involved in melatonin production. Stimulation of β-adrenergic receptors of pinealocytes sets off a cascade of events leading to melatonin production.[11] α-Adrenergic stimulation potentiates the effects of β-receptor stimulation.[12-14] Pinealectomy abolishes plasma levels of melatonin,[15] and there is no substantial evidence for extrapineal production of melatonin in humans.[16] Thus, pineal gland melatonin formation should provide a physiologic marker for noradrenergic activity and potentially could serve as a means for examining the effects of antidepressant treatment on noradrenergic function.

Several investigators have measured plasma melatonin levels in studies of antidepressant treatments using repeated samples over all or part of a 24-hour period.[17-20] By calculating the area under the curve for repeated plasma samples, one can compare melatonin production under different conditions. Alternatively, urinary excretion of the principal metabolite of melatonin, 6-hydroxymelatonin, may provide a convenient means for assessing melatonin production. Approximately 85% of melatonin is metabolized and excreted as the glucuronidated and sulfated conjugates of 6-hydroxymelatonin. By measuring total 6-hydroxymelatonin output in a 24-hour urine sample, one obtains an integrated measure of melatonin formation.[21]

We have applied this tool to our ongoing investigations of the mechanism of action of antidepressant treatments. Previous work from our group has shown that diverse therapies (electroconvulsive; lithium carbonate, the NE reuptake inhibitor desipramine, the serotonin reuptake inhibitor zimelidine, the MAO type A inhibitor clorgiline, and the aminoketone antidepressant bupropion) all reduce whole-body NE output as measured by the summated excretion of NE plus its principal metabolites MHPG, vanillylmandelic acid (VMA), and normetanephrine.[22-26] At the same time cardiovascular functioning is maintained or enhanced.[27] This can be interpreted as reflecting increased "efficiency" in NE functioning following antidepressant therapy; ie, "less" NE is doing the same or more.[27] To test this hypothesis further, we decided to measure 6-hydroxymelatonin excretion and whole-body NE turnover (ΣNE) in depressed patients before and after treatment. If antidepressants in fact increase the "efficiency" of noradrenergic systems, then 6-hydroxymelatonin excretion should be maintained in the face of decreasing total NE production. We report herein our results with three types of antidepressants: monoamine oxidase inhibitors (tranylcypromine sulfate and clorgiline), a tricyclic (desipramine hydrochloride), and a unicyclic aminoketone (bupropion hydrochloride).

SUBJECTS AND METHODS

Twenty-seven patients who fulfilled Research Diagnostic Criteria[28] for major depression were admitted to a clinical research ward at the National Institutes of Health, Bethesda, Md. All patients were placed on a standard low-monoamine, restricted-caffeine diet.[29] Following a drug-free washout period of at least three weeks, baseline 24-hour urine samples (7 AM to 7 AM) were collected into bottles containing 10 mL of a 3% solution of sodium metabisulfite. The urine samples were refrigerated at 4°C during the day of collection. After measuring the volume of each completed collection, aliquots were frozen at −20°C until the time of the assay. Urine volumes in excess of 900 mL were considered acceptable for the study. The lighting conditions on the ward did not vary over the course of the study, and patients did not leave the hospital on the days in which the 24-hour urine samples were collected. Also, patients did not engage in excessive or strenuous physical activity during sample collection days. During sampling periods, if patients were unable to sleep at night, they were encouraged to remain resting in bed.

Under double-blind conditions, patients were assigned to receive one of the following treatments, after we had obtained informed consent: (1) the MAO inhibitor tranylcypromine; (2) the MAO type A inhibitor clorgiline; (3) the tricyclic antidepressant desipramine; or (4) the unicyclic antidepressant bupropion. The seven patients treated with bupropion were drawn from another study,[26] in which ΣNE, but not 6-hydroxymelatonin output, was examined. Dosage was titrated as tolerated under the supervision of a psychiatrist who was not "blind" to treatment. Table 1 lists the final dose achieved as well as demographic and diagnostic data for each patient. Following three to six weeks of treatment, 24-hour urine samples were again obtained using identical procedure as described above. For one subject (B) posttreatment whole-body NE measures were not determined, although 6-hydroxymelatonin concentrations were measured; for a different subject (AA), posttreatment 6-hydroxymelatonin level could not be determined.

All laboratory assays were performed on coded urine samples by personnel who were blind to the clinical status and treatment condition represented by each sample. 6-Hydroxymelatonin was measured using a negative chemical ionization–mass spectroscopic method as previously described.[30] Briefly, an internal standard of tetradeutero–6-hydroxymelatonin sulfate was added to 3 mL of urine, the conjugates were hydrolyzed enzymatically, and the free melatonin metabolites were extracted into dichloromethane. After reaction with *t*-butyldimethylchlorosilone and pentafluoropropionic anhydride, the stable product was partially purified on a silica gel column, and the ratio of deuterated to endogenous 6-hydroxymelatonin derivatives was determined using gas chromatographic–mass spectrometric analysis. The urine samples were assayed for NE and its major metabolites (MHPG, VMA, and normetanephrine) using mass fragmentography.[31] The sum of NE, normetanephrine, MHPG, and VMA output was used to indicate ΣNE.[23] When two or three consecutive daily urine samples could be obtained from a patient during the baseline and/or postantidepressant treatment conditions, mean values for 6-hydroxymelatonin and ΣNE were taken. Baseline and posttreatment samples for each patient were assayed on the same run.

Pretreatment and posttreatment measures were compared using *t* tests for related samples. To determine if the effects of the two MAO inhibitors were different, a Mann-Whitney *U* test was applied. Since clorgiline and tranylcypromine did not have different effects on either ΣNE ($P>.50$) or 6-hydroxymelatonin ($P=.50$), the data from patients receiving MAO inhibitor treatment were pooled for subsequent analyses.

RESULTS

Whole-body NE turnover was significantly reduced following antidepressant treatment, falling from a mean (±SEM) pretreatment level of 38.3±2.1 (6.5±0.4) to 23.8±1.8 μmol/d (4.0±0.3 mg/24 h) ($P=.0001$; Table 1). (A more detailed table showing the effects of treatment on urinary NE and each of its metabolites is available on request.) At the same time, 6-hydroxymelatonin excretion increased from a mean baseline of 7.2±1.2 μg/24 h to a mean posttreatment level of 9.2±1.4 μg/24 h ($P=.04$; Table 1).

The reduction in ΣNE was greatest in patients receiving MAO inhibitor treatment, all of whom demonstrated this effect ($P=.0003$). 6-Hydroxymelatonin excretion increased in eight of ten patients so treated, a trend that did not quite reach statistical significance ($P=.08$). Desipramine also significantly reduced ΣNE ($P=.0009$), with a trend toward increasing 6-hydroxymelatonin excretion ($P=.14$). Bupropion reduced ΣNE ($P<.05$); its effect on 6-hydroxymelatonin was not significant ($P=.80$; Table 2).

COMMENT

These findings confirm previous reports that antidepressant treatments lead to a reduction in ΣNE in depressed patients.[22-26] At the same time, we found that 6-hydroxymelatonin excretion increased. Thus, our study fails to provide evidence for a reduction in noradrenergic function following long-term antidepressant treatments. On the contrary, our findings are consistent with an interpretation that antidepressant therapy leads to an increase in noradrenergic "efficiency," in that functional output as previously

Table 1.—Demographic, Diagnostic, and Treatment Data*

Patient/Age, y/Sex	RDC Diagnosis	Final Dose, mg/d	6-Hydroxymelatonin, μg/24 h		ΣNE, μmol/d (mg/24 h)	
			Baseline	Posttreatment	Baseline	Posttreatment
Tranylcypromine Sulfate						
A/51/F	UP	50	1.2	6.1	42.6 (7.2)	22.9 (3.9)
B/36/M	UP	40	4.8	7.1	...	...
C/34/F	BP-II	40	2.0	2.5	42.9 (7.3)	11.8 (2.0)
Clorgiline						
D/70/F	UP	5	1.5	5.2	23.9 (4.0)	16.0 (2.7)
E/56/M	BP-II	10	26.5	31.2	58.9 (10.0)	29.6 (5.0)
F/30/M	BP-II	15	8.9	14.1	39.6 (6.7)	20.1 (3.4)
G/42/M	BP-I	15	8.2	6.7	67.0 (11.3)	23.3 (3.9)
H/37/F	UP	10	14.9	9.8	33.0 (5.6)	13.2 (2.2)
I/32/M	BP-I	2.5	6.1	10.5	28.4 (4.8)	18.9 (3.2)
J/44/F	BP-I	5	7.3	9.3	39.7 (6.7)	12.8 (2.2)
Desipramine Hydrochloride						
K/29/F	UP	150	7.3	0.8	41.8 (7.1)	16.7 (2.8)
L/48/F	BP-II	250	0.6	6.4	49.2 (8.3)	31.3 (5.3)
M/35/F	UP	200	6.4	1.0	42.0 (7.1)	33.3 (5.6)
N/51/F	BP-II	250	2.5	2.7	34.4 (5.8)	29.7 (5.0)
O/40/F	UP	150	3.3	4.3	29.1 (4.9)	18.8 (3.2)
P/28/F	BP-I	225	11.3	15.9	28.6 (4.8)	16.4 (2.8)
Q/48/F	UP	200	5.9	14.0	28.2 (4.8)	9.2 (1.6)
R/32/M	UP	400	14.8	19.8	39.0 (6.6)	38.1 (6.4)
S/32/F	BP-II	200	3.2	8.1	23.1 (3.9)	18.8 (3.2)
T/69/F	UP	100	11.7	19.3	41.3 (7.0)	19.8 (3.4)
Bupropion Hydrochloride						
U/41/F	UP	300	3.1	5.5	18.6 (3.1)	22.7 (3.8)
V/31/F	UP	400	18.1	12.2	31.4 (5.3)	23.9 (4.0)
W/51/F	BP-II	500	4.5	5.6	51.1 (8.6)	41.1 (7.0)
X/26/M	BP-I	425	6.2	17.2	39.8 (6.7)	40.6 (6.9)
Y/53/F	BP-I	375	6.9	1.3	43.3 (7.3)	28.6 (4.8)
Z/54/M	BP-II	400	1.0	2.0	39.3 (6.6)	33.5 (5.7)
AA/26/F	BP-II	400	...	...	40.7 (6.9)	27.0 (4.6)
41.8±2.3†	...	...	7.2±1.2†	9.2±1.4†	38.3±2.1 (6.5±0.4)†	23.8±1.8 (4.0±0.3)†

*RDC indicates Research Diagnostic Criteria; ΣNE, whole-body norepinephrine turnover; UP, unipolar; BP, bipolar (I and II).
†Mean±SEM.

Table 2.—Drug Effects on 6-Hydroxymelatonin and ΣNE*

Treatment Group	6-Hydroxymelatonin, g/24 h		ΣNE, μmol/d (mg/24 h)	
	Baseline	Post-treatment	Baseline	Post-treatment
MAO inhibitor	8.1±2.4	10.3±2.5	41.8±4.6 (7.1±0.8)	18.7±2.0 (3.2±0.3)
Desipramine hydrochloride	6.7±1.5	9.2±2.3	35.7±2.6 (6.0±0.4)	23.2±2.9 (3.9±0.5)
Bupropion hydrochloride	6.6±2.5	7.3±2.5	37.7±3.9 (6.4±0.7)	31.1±2.9 (5.3±0.5)

*ΣNE indicates whole-body norepinephrine turnover; MAO, monoamine oxidase. Values are mean±SEM.

measured by cardiovascular values[27] and in the present instance by 6-hydroxymelatonin excretion is not only maintained but is possibly enhanced, while at the same time total NE production is decreased.

Could each of the observed antidepressant effects simply reflect the result of drug-induced changes in the metabolism of NE? For example, by decreasing presynaptic reuptake of NE and consequent intraneuronal catabolism, NE metabolite formation could be altered. Also, the reuptake process serves physiologically to protect the pineal gland from nonsynaptic adrenergic stimulation; reuptake blockade can increase the response of pineal *N*-acetyltransferase activity to stimulation by stress.[32] However, drug-induced changes in metabolism are unlikely to explain our findings completely since we included different types of pharmacologic agents with markedly different effects on catecholamine metabolism.

Preclinical studies examining the effects of antidepressant administration on pineal function in animals have yielded apparently conflicting results. King et al[33] reported that MAO inhibition led to an increase in melatonin content of rat pineal gland. Oxenkrug and coworkers[34] also found an increase in rat pineal melatonin content following administration of an MAO type A inhibitor but not with an MAO type B inhibitor; the former effect was greatly diminished by superior cervical ganglionectomy.[35] Heydorn et al,[36] on the other hand, looking at stimulated melatonin output instead of pineal basal content, found that long-term, though not short-term, desipramine or nialamide

exposure reduced both the isoproterenol- and darkness-induced elevation of melatonin in rat pineal gland and serum. The same group, however, has recently reported that long-term desipramine treatment does not decrease nocturnal plasma melatonin concentrations in depressed patients.[18]

Our findings of increased urinary 6-hydroxymelatonin excretion following antidepressant therapy are compatible with this and other recent reports of the effects of antidepressants on plasma melatonin in man. Thompson et al[19] studied six normal subjects and six depressed patients following long-term desipramine treatment. The depressed subjects showed a significant rise in nocturnal melatonin secretion after three weeks of desipramine treatment. Interestingly, the normal subjects did not demonstrate significant differences in pretreatment and posttreatment nocturnal plasma melatonin concentrations. Cowen et al[20] studied ten healthy subjects who were given desipramine and found that short-term administration led to a significant increase in mean midnight plasma melatonin concentrations. This increase peaked on the fifth day of desipramine administration and then returned toward pretreatment levels with no significant difference between baseline and 19-day treatment values. Murphy et al[37] examined the effects of three different MAO inhibitors on morning plasma melatonin levels in depressed patients. Both clorgiline and tranylcypromine, the MAO type A inhibitor and the nonselective MAO inhibitor used in our study, increased morning plasma melatonin concentrations in depressed patients following long-term (three-week) treatment; the MAO type B inhibitor deprenyl (selegiline), in contrast, did not increase plasma melatonin levels, suggesting that inhibition of MAO type A by either a selective or a nonselective MAO inhibitor can increase pineal melatonin output. Mendlewicz et al[17] reported that in four depressed patients, mean plasma melatonin values and day-night differences were similar before and after four weeks' treatment with amitriptyline. In the only other published study examining the effects of antidepressant treatment on urinary excretion of 6-hydroxymelatonin, Sack and Lewy[38] reported that in four depressed patients, desipramine treatment led to an increase in 6-hydroxymelatonin output, which was sustained over three weeks of treatment.

Despite the overall general agreement, it is not clear from the studies of either plasma melatonin or urinary 6-hydroxymelatonin that a stable, reproducible drug-induced increment can be shown after treatment with MAO inhibitors (including type A inhibitors) and tricyclic antidepressants. Our sample size for each treatment group is relatively small, and the increase in 6-hydroxymelatonin excretion following antidepressant treatment reaches statistical significance only when the three treatment groups, including bupropion, are pooled. At the time that this study was designed we believed that including bupropion as one of the treatments would enable us to look at the effects on noradrenergic functioning of a drug that did not have direct biochemical effects on this system. Though bupropion is characterized preclinically as a dopamine uptake inhibitor without appreciable effects on NE reuptake or on MAO,[6] one of the drug's principal metabolites in man, hydroxybupropion, may in fact be an NE reuptake inhibitor.[39] It is possible, therefore, that bupropion, through its active metabolite, is exerting desipraminelike effects on NE uptake.[40]

Even though the antidepressant-induced changes in melatonin measures are not always robust and in the same direction, our studies do reveal remarkably consistent treatment-associated reductions in whole-body NE output. Only two of 27 patients (patients U and X) failed to show the reduction in ΣNE (Table 1). Since the same urine samples were used to measure output of both total NE and 6-hydroxymelatonin, it is of interest to assess the relationship between the factors. In these patients, however, all of whom were depressed during the baseline measure, no correlation could be identified using standard parametric and nonparametric approaches. Thus, although melatonin production may be extensively controlled by the level of stimulation of β-receptors on the pineal gland, it does not appear to relate appreciably to total NE output in depressed patients in any direct manner.

These findings suggest caution in using melatonin output as a functional NE measure. It might be argued on the basis of the model discussed in the introduction that the variation in melatonin or 6-hydroxymelatonin is somehow more directly related to brain noradrenergic function than is urinary NE output. However, pineal melatonin production is not a measure of central noradrenergic function. The pineal gland lies outside of the blood-brain barrier[41]; it can be affected by circulating catecholamines when reuptake is blocked.[32] Its innervation is via the superior cervical ganglion, and its activity does not necessarily parallel that of other noradrenergic systems in the brain. Also arguing against melatonin production being more directly related to central noradrenergic function than is urinary NE output is an extensive animal literature showing that antidepressants produce consistent reductions in central nervous system β-adrenergic receptors[8,9]; the concomitant finding in man is the reduction of total NE output.[42] We believe that this reduction must be of central origin. Tricyclics can *increase* plasma NE levels,[43,44] presumably through reduction in clearance (which has been demonstrated in humans[45]), despite a reduction in total output.[22] Monoamine oxidase inhibitors, in humans, reduce plasma NE levels,[46,47] but in the absence of central influences in the rat (ie, following "pithing"), they too can actually increase plasma NE levels.[48] These findings are compatible with the observations that locus ceruleus firing in the central nervous system of rodents is markedly reduced after both tricyclic and MAO inhibitor administration.[49,50]

Plasma melatonin and urinary 6-hydroxymelatonin measures are, however, useful in demonstrating that no matter how great the antidepressant-induced down regulation of β-receptors, the output is not reduced. If anything, chronic antidepressant use enhances melatonin output. This is compatible with Stone's[51] suggestion that drug effects such as down regulation of β-receptors may be partially compensatory to and of less functional significance than the opposing forces of uptake inhibition and MAO inhibition. These findings support the theory that antidepressant treatment leads to increased "efficiency" in noradrenergic systems with an enhancement or maintenance of function and a decrease in total NE production.

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