DEXAMETHASONE SUPPRESSION TEST IDENTIFIES A SUBSET OF ELDERLY DEPRESSED PATIENTS WITH REDUCED PLATELET SEROTONIN TRANSPORT AND RESISTANCE TO IMIPRAMINE INHIBITION OF TRANSPORT

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Dysregulation of the hypothalamus-pituitary-adrenal axis (HPA) is more common in elderly patients with depression than in younger depressed patients, and glucocorticoids are known to influence serotonergic function. Elderly depressed patients are also reportedly more resistant to therapeutic effects of antidepressants. In the current study, we measured platelet serotonin transporter binding sites and transport function in young and elderly depressed patients and determined the relationship to HPA status as assessed with the dexamethasone suppression test (DST). The density and affinity of transporter molecules showed no differences between young and elderly depressed patients, regardless of DST results. Nevertheless, transporter function showed a substantial interaction of aging with DST: elderly DST suppressors showed a deficit in [^{3}H]serotonin uptake capabilities and resistance to imipramine inhibition of uptake. No such defects were seen in the young depressed cohort, regardless of DST status, nor in elderly depressed DST non-suppressors. These results are consistent with the view that depression in the elderly exhibits basic biological differences from depression in earlier life, and that such distinctions may account in part for therapeutic ineffectiveness of antidepressants in specific subgroups, associated with the presence or absence of appropriate HPA regulation. Depression and Anxiety 6:19–25, 1997.

Key words: *aging; platelet serotonin transporter function in depression; depression; imipramine; tricyclic antidepressants*

INTRODUCTION

Elevated cortisol concentrations and impaired hypothalamus-pituitary-adrenal axis (HPA) regulation are characteristic of depression (Carroll et al., 1976; Pfohl et al., 1985), as evidenced by the high frequency of abnormal dexamethasone suppression test (DST) results (Carroll et al., 1981). Aging also produces an increased incidence of abnormal DSTs (Stangl et al., 1986; Sharma et al., 1988) an association that is even more pronounced in elderly depression (Ritchie et al., 1990b; Brodaty et al., 1991). The close interrelationship between glucocorticoid status and serotonergic function (Chan and Lee, 1985; Arora and Meltzer, 1986; Kitayama et al., 1988; Shimoda et al., 1988; Joëls and De Kloet, 1991; Pepin et al., 1992) suggests that HPA dysregulation may have a significant impact on the reactivity to tricyclic antidepressants and particularly the serotonin-specific reuptake inhibitors that have become the mainstay of depression therapy (Carroll et al., 1981; Aghajanian et al., 1993; Young, 1994). Central serotonergic pathways are, in turn, major contributors to HPA control (Stokes et al., 1987;

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Abbreviations: ANOVA, analysis of variance; DST, dexamethasone suppression test; HPA, hypothalamus-pituitary-adrenal axis.

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Korte et al., 1991) and, again, this regulation is disrupted in depression (Koenig et al., 1987; Lesch et al., 1990; Maes et al., 1991). It is, thus, of key importance that resistance to tricyclic antidepressants is a hallmark of elderly depression (Danish University Antidepressant Group [DUAG], 1986, 1990; Roose et al., 1994; Nelson et al., 1995) and, in fact, platelets derived from elderly depressed patients show that imipramine is less effective in blocking serotonin uptake than in elderly controls, young controls, or young depressed patients (Slotkin et al., 1989).

These findings all indicate the likelihood that there are basic biological differences in elderly depression that influence the serotonin transporter and/or its reactivity to antidepressants and that some of these differences may reside in the status of HPA regulation. Recent work in animal models of aging and glucocorticoid excess also suggests that these two variables have a significant impact on the expression and function of the transporter, both in the central nervous system and platelets (Fumagalli et al., 1996; Slotkin et al., 1996, 1997). Accordingly, the current study examines the relationship of DST status and aging to platelet serotonin transporter function in depressed patients.

METHODS

Patients were recruited under the auspices of the Duke University Mental Health Clinical Research Center for the Study of Depression in the Elderly. All patients received a structured interview (Duke Depression Evaluation Schedule for the Elderly) derived from the Diagnostic Interview Schedule and the Mini-Mental State Examination. Data were then reviewed by a diagnostic team and a standardized DSM-III diagnosis was assigned to each subject. Those selected for this study fulfilled DSM-III criteria for major depression and were free of all psychotropic medications for at least 7 days, but usually longer; over 75% of the patients had never received tricyclic antidepressants or benzodiazepines prior to the study. There were also no significant differences in any of the variables between those receiving medication prior to the drug-free period and those who did not receive prior medication (data not shown). All patients with any medical condition that might contribute to depression of dementia or who had any major medical disorder, such as diabetes, cardiovascular or pulmonary disease, or cancer, were excluded. The patients were subdivided into DST suppressors and non-suppressors (see below) and into two age categories: young (<50 years old) and elderly (>60 years old). The young cohort averaged 34 ± 1 years of age (33 ± 2 for DST suppressors, 36 ± 4 for non-suppressors) whereas the elderly cohort averaged 67 ± 2 years (68 ± 3 for suppressors, 66 ± 6 for non-suppressors). Blood samples for studies of platelet uptake and binding mechanisms were obtained with standard ACD Vac-u-Tainer tubes between 8:30 and 10:00 AM and preceded evaluation of the DST.

PLATELET PREPARATION AND [^{3}H]SEROTONIN UPTAKE MEASUREMENTS

Platelet-rich plasma was isolated by serial centrifugation (Slotkin et al., 1991). Blood was first sedimented at 100*g* for 10 min and the plasma layer removed; this process was repeated at 100*g*, 250*g*, and 600*g*. The platelet-rich plasma fractions were then pooled and diluted with one-half volume of calcium-free Krebs-Henseleit medium (calcium omitted and replaced with 2 mM EDTA) and sedimented at 3,000*g* for 5 min. The washed platelet pellet was then gently resuspended in 4 ml of calcium-free Krebs-Henseleit medium using a smooth glass homogenizer fitted with a Teflon pestle and an aliquot removed for counting of platelets.

For determination of [^{3}H]serotonin uptake, samples were prepared containing 20 µl of the platelet preparation and final concentrations of 0.1, 0.3, or 1 µM serotonin [1,2-^{3}H(N)] creatinine sulfate (specific activity, 21.5 Ci/mmol, New England Nuclear Corp., Boston, MA; isotopically diluted to a final specific activity of 2.7 Ci/mmol with unlabeled serotonin creatinine sulfate, Sigma Chemical Co., St. Louis, MO) in 1 ml of calcium-free Krebs-Henseleit medium, with or without the addition of 10 µM imipramine HC1 (Ciba-Geigy, Summit, NJ). Incubations lasted for 10 min at 37°C, after which time the platelets were harvested by rapid vacuum filtration on glass fiber filters. The incubation tubes were washed with 10 ml of 150 mM NaCl + 2 mM EDTA and the wash was passed through the filter apparatus, after which the filters were counted for the radiolabel. Analysis of serotonin (HPLC with electrochemical detection; data not shown) before and after incubation indicated no significant release of endogenous material from the platelets and confirmed the lack of degradation of serotonin in the medium (Lau et al., 1985; Slotkin et al., 1986). Specific uptake was determined as the difference between samples without and with imipramine, and under these conditions uptake was linear with tissue concentration (up to at least 5 times the number of platelets used) and incubation time (up to at least 20 min).

Every patient sample was run concurrently with a standard preparation of rat platelets (adult female Sprague-Dawley rats; Zivic-Miller Laboratories, Allison Park, PA) to validate the uptake assay and to verify the inhibitory potency of imipramine (Slotkin et al., 1986).

PLATELET MEMBRANE PREPARATION AND [^{3}H]IMIPRAMINE BINDING MEASUREMENTS

Platelet-rich plasma was sedimented at 16,000*g* for 10 min and the pellet was resuspended (Polytron, Brinkman Instruments, Westbury, NY) in 50 mM Tris + 150 mM NaCl + 20 mM EDTA, pH 7.5. After sedimentation at 39,000*g* for 10 min, the platelet pellet was resuspended in hypotonic medium (5 mM Tris

+ 5 mM EDTA, pH 7.5), sedimented at 39,000*g*, suspended in 70 mM Tris (pH 7.5), resedimented, and finally resuspended in the assay buffer (50 mM Tris + 120 mM NaCl + 5 mM KC1, pH 7.5). Aliquots were taken for protein determinations (Lowry et al., 1951) and the platelet aliquots were frozen and maintained at –70°C; preliminary studies found no difference in platelet [^{3}H]imipramine binding parameters after freezing and thawing (Nemeroff et al., 1988).

Binding of [^{3}H]imipramine (specific activity, 45.5 Ci/mmol; New England Nuclear) to platelet membrane preparations was determined by methods described in detail previously (Nemeroff et al., 1988). Briefly, seven concentrations were used (0.25 to 8 nM) spanning the K_d (approximately 1.5 nM). Each assay was conducted in triplicate using ~100 µg of platelet protein in a final volume of 250 µl of assay buffer. Tubes were incubated on ice for 60 min after which 5 ml of ice-cold buffer was added and the labeled membranes harvested by vacuum filtration. Non-specific binding (less than 20% of total binding) was determined as binding in the presence of 100 µM desmethylimipramine (Marion Merrell Dow Pharmaceuticals, Cincinnati, OH). Scatchard analyses used to determine the K_d and B_{max} of [^{3}H]imipramine binding were fitted by linear regression. Assays with a correlation coefficient of <0.90 were excluded. The potential confounding influence of seasonal variation in B_{max} values was evaluated and found to be insignificant (data not shown).

DEXAMETHASONE SUPPRESSION TEST

Subjects received a 1 mg overnight DST after screening for the usual factors that can invalidate test results (Carroll et al., 1981). Inpatients had three blood samples drawn for post-dexamethasone plasma cortisol at 0800, 1500 and 2200 h. Outpatients had a single blood sample drawn at 1500 hr (Carroll et al., 1981). Any plasma cortisol (Ritchie et al., 1990a) greater than 5 µg/dL (138 nM) classified a subject as a non-suppressor. We have shown separately that in elderly depressed patients there is no difference in plasma dexamethasone concentrations between suppressors and non-suppressors (Ritchie et al., 1990b).

DATA ANALYSIS

Results are presented as means and standard errors with significant differences between groups established by multivariate ANOVA (data log transformed whenever the variance was heterogeneous), using factors of age group, DST status, and serotonin concentration where appropriate. Because of the absence of significant interactions of sex with the other variables (data not shown), results are presented for males and females combined. Significance was assumed at the level of $P < 0.05$.

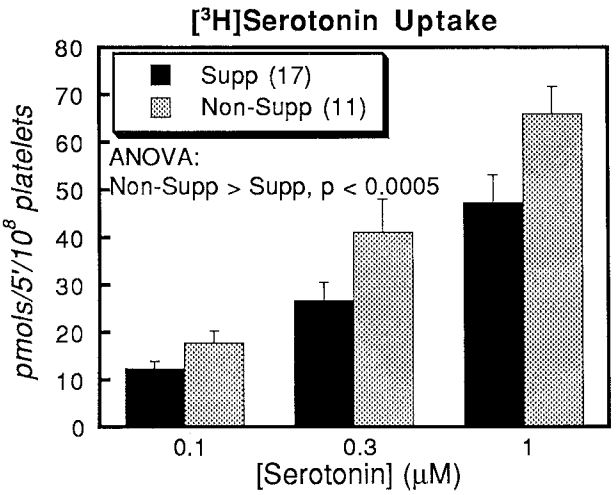
A larger patient population was available for measurement of [^{3}H]imipramine binding than for [^{3}H]serotonin uptake, as binding in frozen membrane preparations could be assessed in batches after storage, whereas uptake required immediate determinations in intact platelets. However, every patient receiving assessment of [^{3}H]serotonin uptake was also represented in the binding studies, and there was no difference between values in this subpopulation and those whose uptake was not evaluated (data not shown). Patients in the binding studies had the same age profile as those in the uptake studies.

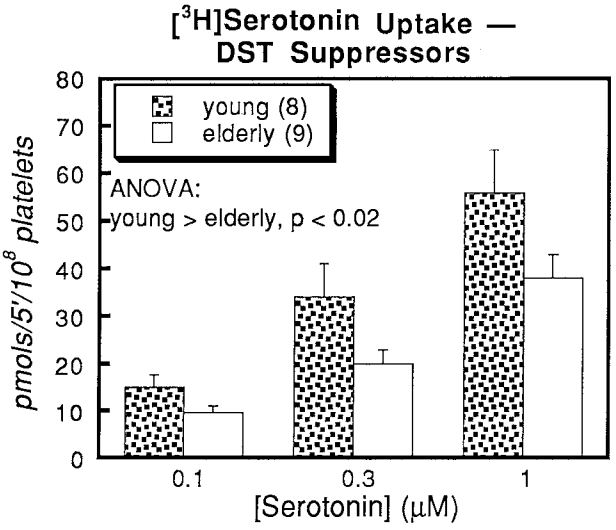
RESULTS

Uptake of [^{3}H]serotonin into intact platelets indicated clear-cut saturation, as increasing the serotonin concentration tenfold (0.1 to 1 µM) produced only a fivefold increase in uptake (Fig. 1). Across the entire concentration range, DST non-suppressors exhibited significantly higher uptake values than did suppressors. This effect was age-dependent, as there was a significant interaction of age × DST, and accordingly the results were compared across age separately for DST suppressors and non-suppressors. Among the suppressors, platelets from young depressed patients showed substantially higher serotonin uptake than did those from elderly depressed patients. In contrast, the non-suppressor population showed no age-dependent differences. Also, comparison within age groups indicated that elderly suppressors had lower values than did elderly non-suppressors, whereas young suppressors and young non-suppressors were indistinguishable from each other.

One of the characteristics of platelet serotonin uptake in elderly depressed patients is a reduced potency of imipramine toward inhibition of [^{3}H]serotonin uptake (Slotkin et al., 1989). Accordingly, we assessed the effects of a submaximally effective concentration of imipramine (40 mM) that has been shown previously to discriminate the resistant population from those that respond normally (Slotkin et al., 1989). When both young and elderly groups were combined, there was no significant difference between DST suppressors and non-suppressors in the ability of 40 nM imipramine to inhibit the uptake of 0.1 µM [^{3}H]serotonin: in both groups, about 30% of the uptake remained at this imipramine concentration (Fig. 2). However, again there was a significant age × DST interaction so that we examined the data divided into separate age × DST bins. In this case, age-dependent differences again emerged in the DST suppressor population, with significant imipramine resistance in the elderly suppressors. No age-dependent differences were seen in the non-suppressor group. Imipramine resistance was not an artifact of the inherent reduction in overall [^{3}H]serotonin uptake in elderly suppressors: within each subgroup, uptake did not correlate significantly with imipramine inhibition, nor did multivariate regression indicate a significant overall relationship between the two variables (data not shown).

Differences in serotonin uptake and imipramine





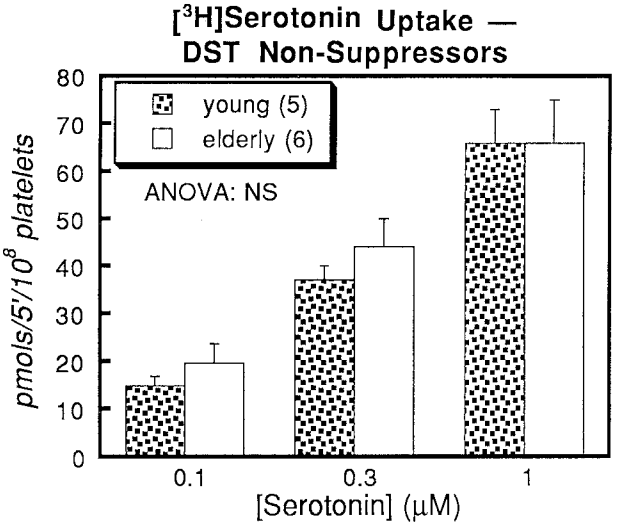


Figure 1. [^{3}H]Serotonin uptake into platelets obtained from depressed patients. Top: Compares DST suppressors (Supp) and non-suppressors (Non-Supp) across both age groups. Middle, Bottom: Subdivide the results into young (<50 years old) and elderly (>60 years old) cohorts. Data represent means and standard errors of the number of patients shown in parentheses. ANOVA across the three serotonin concentrations appears within each panel. Comparisons at individual serotonin concentrations were not conducted because of the absence of interactions of DST × concentration, age × concentration, or DST × age × concentration. In addition, across all three concentrations, ANOVA indicates a significant interaction of age × DST ($P < 0.05$), and elderly suppressors show lower values than elderly non-suppressors ($P < 0.0001$), whereas young suppressors and young non-suppressors are indistinguishable from each other ($P > 0.3$).

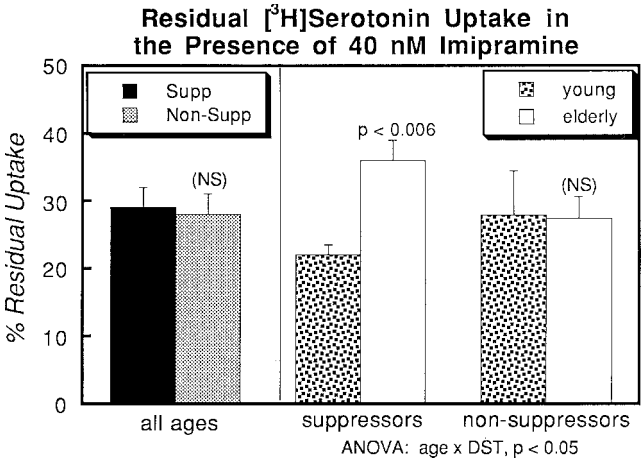


Figure 2. Residual [^{3}H]serotonin uptake (at 0.1 μM serotonin) in the presence of 40 nM imipramine. Left: Compares DST suppressors (Supp) and non-suppressors (Non-Supp) across both age groups. Right: Subdivides the results into young (<50 years old) and elderly (>60 years old) cohorts. Data represent means and standard errors obtained from the same patient populations shown in Figure 1. ANOVA for DST suppression × age appears at the bottom and individual comparisons (Fisher's Protected Least Significant Difference) are shown for each subgroup.

resistance could represent change in transporter function, or alternatively in the concentration or conformation of the transporter itself. To test the latter possibilities, we assessed the number of transporter molecules and their affinity for [^{3}H]imipramine (Fig. 3). There were no differences in either transporter B_{max} or K_d between DST suppressors and non-suppressors, nor were there any significant effects when the population was divided into separate age × DST bins.

Although the sample size used for the [^{3}H]serotonin uptake studies was too small to enable us to discern an increase in the incidence of DST non-suppression in elderly depressed patients, the larger cohort used for the [^{3}H]imipramine binding studies had sufficient power to detect a difference from younger depressed patients. In the elderly group, the incidence of non-suppression was 60%, more than double the rate of 21% seen in the young depressed patients ($P < 0.002$ by Fisher's Exact Test). These results are in keeping with earlier findings (Ritchie et al., 1990b).

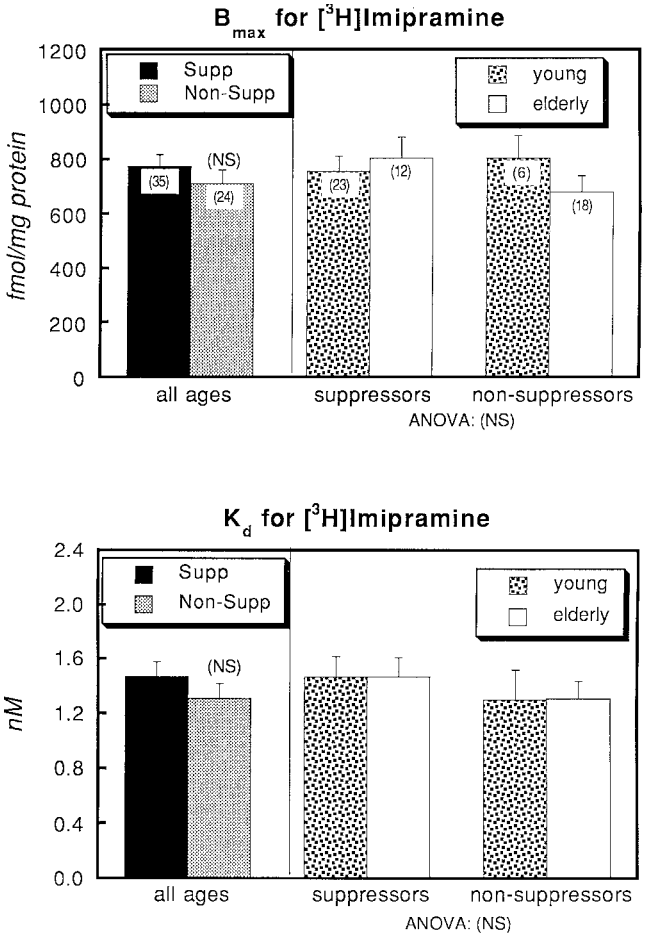


Figure 3. Capacity (B_{max}) and affinity (K_d) of [^{3}H]imipramine binding to platelet membranes, assessed by Scatchard determinations for each patient. Data represent means and standard errors obtained from the number of patients shown in parentheses. ANOVA for DST suppression × age appears at the bottom and individual comparisons are shown within the panels.

DISCUSSION

Results obtained in this study support the hypothesis that elderly depression exhibits basic biological differences from depression earlier in life, specifically targeting the functioning of the serotonin transporter. In previous work, we found that platelet serotonin uptake is decreased in the elderly (Slotkin et al., 1989). The present results confirm this finding and extend it to demonstrate that the differences involve a specific subgroup, elderly depressed patients who exhibit DST suppression; although the population studied was relatively small, nevertheless the differences were significant and robust: approximately 40% reduction in elderly suppressors compared to young suppressors or non-suppressors or to elderly non-suppressors. In contrast, young depressed patients showed no difference in uptake between suppressors and non-suppressors. Thus, a specific combination of aging and DST responsiveness is required in order for abnormalities of serotonin transport to be present. The same factors combine to produce resistance to imipramine inhibition of transport. Previously, it was shown that resistance is detected only in elderly depressed patients, not in elderly controls nor in young depressed patients (Slotkin et al., 1989). We now report that this effect involves specifically the elderly depressed cohort exhibiting DST suppression; no differences were seen between non-suppressors in the young depressed vs. elderly depressed cohorts.

It is especially notable that neither the deficiency in serotonin transport nor the resistance to imipramine noted in the elderly, depressed DST-suppressor group reflected changes in the number of serotonin transporter sites or its affinity for imipramine. Again, these findings are consonant with previous work on elderly depression that did not separate the groups by DST status (Nemeroff et al., 1988; Slotkin et al., 1989); the present results indicate that the overall effect is actually restricted to the suppressor subpopulation. Thus, whereas the actual number of platelet serotonin transporter molecules and, hence, [^{3}H]imipramine binding are definitively reduced in depression regardless of age (Nemeroff et al., 1988), there is no additional difference imposed by age alone or in combination with DST status.

The difference between measures of transporter function, which differed in the elderly DST-suppressors, as compared to transporter numbers/affinity, which did not, points out that vital differences can be missed when only passive binding markers are examined. Indeed, factors such as the energetics of transport, efficiency of intracellular trapping of serotonin in storage vesicles, and transporter efficiency are all variables that can be altered independently of transporter numbers and that are affected in vivo under conditions such as aging or treatment with glucocorticoids or antidepressants (Slotkin et al., 1986, 1996, 1997). As just one example, functional effects on biogenic amine systems that are unique to aging can be elicited through changes in membrane phospholipid composition that affect coupling of receptor or transporter proteins to effectors downstream from the combination of ligand with protein (Benediktsdottir et al., 1995), or that promote affinity changes directed only toward agonist (in this case, serotonin) and not inhibitor (imipramine) molecules (Davies and Lefkowitz, 1981, 1984). Such effects would influence serotonin uptake and imipramine resistance of uptake without necessitating comparable changes in inhibitor binding characteristics and without altering the actual number of transporter molecules.

The key question is whether the differences in platelet serotonin transporter function associated uniquely with elderly depressed patients also occurs within their central nervous system. Unfortunately, changes in transporter function, which require metabolically intact tissue, cannot be evaluated in standard postmortem preparations (as used for passive markers

such as ligand binding) but instead require obtaining intact, "live" synaptosomes from a rapid autopsy, no longer than 30–60 min postmortem (Slotkin et al., 1990; Bissette et al., 1996). Nevertheless, several features of the changes in platelet transporter function have distinct parallels to clinical observations and to animal models. First, if the same changes occur in the central nervous system, then loss of transporter function and resistance to tricyclic antidepressants such as imipramine should occur ordinarily in elderly depression; in fact, a number of studies find that antidepressants are indeed less effective with aging (Danish University Antidepressant Group, 1986, 1990; Roose et al., 1994; Nelson et al., 1995). Given the higher rate of HPA abnormalities in the elderly (Branconnier et al., 1984; Oxenkrug et al., 1984), and particularly with depression (Ritchie et al., 1990b; Brodaty et al., 1991), we would predict a better success rate in the non-suppressor subgroup. Indeed, there is supporting evidence from controlled clinical trials that DST suppressors have a very low rate of specific response to tricyclic antidepressants (approximately 10% above placebo response rates) whereas DST non-suppressors have a specific response rate of approximately 60% because they have an extremely low rate of placebo response (Carroll, 1989). A corollary prediction would be that selective serotonin reuptake inhibitor antidepressant drugs will be especially ineffective in elderly depressed patients with normal DSTs because these drugs are also less effective in their actions on the norepinephrine transporter, which is an additional pharmacodynamic action of imipramine and its primary metabolite, desipramine. These issues aside, we did find a substantial degree of overlap in uptake and imipramine inhibition parameters between DST suppressors and non-suppressors, so that it is unlikely that these measurements alone would provide an ironclad diagnostic test to predict antidepressant efficacy; rather the differences point to basic biological divergence in DST suppressor and non-suppressor subpopulations of elderly depressed patients that predispose them, on the average, to poor antidepressant responsiveness.

A final issue is why non-suppressor status prevents the deterioration of transporter function and imipramine responsiveness in the elderly depressed cohort. Apparently, the loss of uptake and development of imipramine resistance will occur ordinarily in elderly depression but these effects can be offset by the elevation in glucocorticoids or by disruption of the diurnal rhythm associated with HPA dysregulation. The possibility also exists that transporter changes and adrenocortical abnormalities may simply be correlated, but not mechanistically related variables. However, recent studies of chronic glucocorticoid excess in young vs. aging rats provide evidence that there is such a connection (Slotkin et al., 1996, 1997). Long-term administration of dexamethasone produces a decrease in platelet transporter expression and in central nervous system transporter function in young rats but in aged rats the same treatment causes slight increases in platelet expression; additionally, aging itself is associated with increased transporter expression in the brain (Fumagalli et al., 1996; Slotkin et al., 1997) so that changes in function evoked by dexamethasone would likely be less notable than in younger animals (i.e., offsetting of the effects of aging by elevated glucocorticoids). If the same differences pertain to young vs. elderly depression in man, then the aged DST suppressor population would lose transporter function and imipramine sensitivity as a consequence of the combined effects of aging and depression, whereas the comparably aged non-suppressor cohort would have the effect compensated by increased glucocorticoid levels.

In conclusion, we have found basic biologic differences in platelet serotonin transporter function in elderly depression that are specific to patients that exhibit a normal DST and that spare the comparably aged non-suppressor population. Because a relatively small population was studied (n = 28 for uptake parameters; n = 59 for binding parameters), this should be regarded as an initial finding, requiring extension and confirmation in future work; however, even with these limitations, the effects were robust and highly statistically significant. The differences, a reduction in transporter efficiency and in imipramine sensitivity without alterations in the number of transporter sites, are likely to contribute to the relative therapeutic ineffectiveness of antidepressants in elderly patients. These results also point out the complexity of regulation of serotonergic function operating in the interaction of aging and depression, that may only be resolved by assessment of active processes in the synaptic dynamics of serotonin, rather than passive markers such as ligand binding.

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