Cholinergic modulation of the cerebral metabolic response to citalopram in Alzheimer’s disease

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Abstract

Pre-clinical and human neuropharmacological evidence suggests a role of cholinergic modulation of monoamines as a pathophysiological and therapeutic mechanism in Alzheimer’s disease. The present study measured the effects of treatment with the cholinesterase inhibitor and nicotinic receptor modulator, galantamine, on the cerebral metabolic response to the selective serotonin reuptake inhibitor, citalopram. Seven probable Alzheimer’s disease patients and seven demographically comparable controls underwent two positron emission tomography (PET) glucose metabolism scans, after administration of a saline placebo infusion (Day 1) and after citalopram (40 mg, IV, Day 2). The scan protocol was repeated in the Alzheimer’s disease patients 2 months after titration to a 24 mg galantamine dose. At baseline, cerebral glucose metabolism was reduced in Alzheimer’s disease patients relative to controls in right middle temporal, left posterior cingulate and parietal cortices (precuneus and inferior parietal lobule), as expected. Both groups demonstrated acute decreases in cerebral glucose metabolism after citalopram to a greater extent in the Alzheimer’s disease patients. In the patients, relative to the controls, citalopram decreased glucose metabolism to a greater extent in middle frontal gyrus (bilaterally), left middle temporal gyrus and right posterior cingulate prior to treatment. Galantamine treatment alone increased metabolism in the right precuneus, right inferior parietal lobule and right middle occipital gyrus. In contrast, during galantamine treatment, citalopram increased metabolism in the right middle frontal gyrus, right post-central gyrus, right superior and middle temporal gyrus and right cerebellum. The combined cerebral metabolic effects of galantamine and citalopram suggest, consistent with preclinical data, a synergistic interaction of cholinergic and serotonergic systems.

Keywords: Alzheimer’s disease; positron emission tomography (PET); acetylcholine; serotonin; citalopram; galantamine

Abbreviations: ADAS-COG = Alzheimer’s disease Assessment Scale score; MCI = mild cognitive impairment; MMSE = mini mental status examination; NPI = Neuropsychiatric Inventory; PET = positron emission tomography
Introduction

The pre-synaptic cholinergic deficit has been a major focus of research and treatment development in Alzheimer's disease (Davies and Maloney, 1976). Deficits in other neurotransmitters, including monoamine systems (dopamine, serotonin and norepinephrine) have been reported, as well (Palmer and DeKosky, 1993). In particular, serotonergic deficits (decrease in transporters and 5-HT1A and 5-HT2A receptors), as shown by neuropathological and neuroimaging studies, have been shown to be greater and more widespread than other neurotransmitter deficits in Alzheimer's disease, including other monoaminergic and muscarinic cholinergic systems and have also been observed in mild cognitive impairment (MCI, as reviewed by Cross et al., 1993). In particular, serotonergic deficits (decrease in transporters of the two systems in memory function (Azmitia and Segal, 1978; Azmitia et al., 1988; Garcia-Alloza et al., 2005). Combined administration of cholinergic and serotonergic antagonists or synthesis inhibitors produced greater cognitive deficits than administration of either compound alone (Vanderwolf, 1987; Little et al., 1995). Combined enhancement of cholinergic and serotonergic function has shown relative greater improvements in memory (Altman et al., 1987).

The purpose of the present study was to evaluate whether chronic treatment with cholinesterase inhibitors would affect the cerebral metabolic response to citalopram in Alzheimer's disease patients. Galantamine was the cholinesterase inhibitor chosen for use in the study because it is an effective and well-tolerated medication (Raskind et al., 2000; Tariot et al., 2000). Galantamine is a competitive and reversible inhibitor of acetylcholinesterase that also allosterically modulates the nicotinic acetylcholine receptor (Thomsen and Kewitz, 1990; Schrattenholz et al., 1996). The dual mechanisms of action might result in a greater net effect on serotonin systems (as well as other monoamine systems) as compared with cholinesterase inhibitors with a single mechanism of action. The acute cerebral metabolic response to the selective serotonin reuptake inhibitor (SSRI), citalopram was measured as has been done in previous studies (Smith et al., 2002a, b). Citalopram was chosen as it is the most pharmacologically selective of the SSRI's, is available in intravenous form and is well tolerated in individuals across the lifespan (Goldberg et al., 2004). To measure the dynamic response of the serotonin system, the most direct method would be to measure changes in serotonin receptor availability secondary to a pharmacologic increase in serotonin using a similar paradigm as has been developed for the dopamine system (Dewey et al., 1993). As has been reviewed previously (Smith et al., 2002a, b), the available serotonergic receptor radiotracers do not show an interpretable changes in specific binding associated with a pharmacologic increase in serotonin. Thus, the combination of the glucose metabolism measures with acute intravenous administration of citalopram was used as a measure of the functional response to acute serotonin transporter occupancy (>70%; Hinz et al., 2008) and a secondary, pharmacologic increase in serotonin (Kreiss et al., 1993). The paired positron emission tomography (PET) scans were performed in the Alzheimer's disease patients before and during treatment with galantamine and in the control subjects on one occasion. There were two aims of the study. Aim 1 was to compare the cerebral metabolic response to citalopram in Alzheimer's disease patients relative to controls. The hypothesis was tested that the cerebral metabolic response to citalopram would be greater in Alzheimer's disease patients relative to controls. Aim 2 was to compare the cerebral metabolic response to citalopram in the Alzheimer's disease patients before and during galantamine treatment. The hypothesis was tested that the cerebral metabolic response to citalopram in the Alzheimer’s disease patients would be enhanced by galantamine treatment, due to the synergistic interaction between the cholinergic and serotonergic systems shown in preclinical studies.

Materials and methods

Alzheimer’s disease patients and controls underwent medical (including laboratory testing and toxicology screening), psychiatric evaluation (SCID) and MRI (GE 1.5T Magnetom Vision). Subjects were excluded based upon a history of or current significant medical (including insulin dependent diabetes), psychiatric (DSM-IV axis I psychiatric disorder) or neurological disorder (except for Alzheimer’s disease in the patients), substance abuse or use of prescription or over the counter medications with central nervous system effects (including cholinesterase inhibitors, antihistamines, cold medications) within the past month. Seven patients who met DSM-IV and National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related
Disorders Association criteria (McKhann et al., 1984) for probable Alzheimer’s disease were enrolled in the study (mean age 76.3 ± 11.1 years, two males/five females, education 14.6 ± 2.8, mini mental status examination (MMSE; Folstein et al., 1975) score 23.4 ± 1.9). The total Alzheimer’s disease Assessment Scale score (ADAS-COG, long form; Rosen et al., 1984) was 19.3 ± 7.2. The total Neuropsychiatric Inventory (NPI; Cummings et al., 1994) score at baseline was 7.7 ± 10.9. Six of the seven patients had never been treated with a cholinesterase inhibitor. Seven healthy controls were recruited from the community and enrolled in the study (mean age 71.9 ± 6.2 years, two males/five females, education 14.0 ± 2.5, MMSE score 29.1 ± 0.9). After a complete description of the study to the subjects, written informed consent was obtained according to procedures established by the Institutional Review Board and the Radiation Safety Committee of the North Shore-Long Island Jewish Health System.

To address Aim 1 of the study, controls and Alzheimer’s disease patients underwent one PET scan session to measure the cerebral metabolic effects of citalopram. To address Aim 2 of the study, galantamine treatment began in the Alzheimer’s disease patients after the baseline PET scan session and the PET scans were repeated after 8 weeks of treatment at the highest galantamine dose (week 16). The treatment protocol involved 4 weeks of administration of galantamine at a dose of 8 mg per day, followed by 4 weeks at 16 mg and then an increase to 24 mg, if clinically indicated as in previous clinical trials (Raskind et al., 2000; Tariot et al., 2000). All patients tolerated the galantamine well and were titrated to the 24 mg dose of galantamine. Subjects were treated for an additional 2 months after the second PET scan session on the 24 mg dose for a total of 24 weeks (4 months at the 24 mg dose). The clinical and neuropsychological assessments performed at the time of the two PET scan sessions (week 16) and the end of the treatment study included the MMSE, NPI, Clinician’s Interview-Based Impression of Change plus Caregiver Input (CIBIC-plus; Schneider et al., 1997) and the ADAS-COG. The clinical and neuropsychological data were analysed using repeated measures analysis of variance (ANOVA).

Serum and plasma samples for assays of citalopram levels and prolactin concentrations, respectively, were obtained at pre-determined intervals (pre-infusion, end of infusion and 15, 30 60, 90, 120 min post-infusion). Prolactin concentrations were measured to evaluate the effects of citalopram administration on the serotonin system independently of the glucose metabolism measures. The acute increase in prolactin after a pharmacologic increase in serotonin has been reported to reflect an activation of post synaptic, hypothalamic serotonin receptors (5-HT1A, 5-HT2A and 5-HT2C subtypes; Raap and Van de Kar, 1999). The assays were performed in the Geriatric Psychopharmacology Laboratory, Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine (Smith et al., 2002a, b). The ELISA measurements for citalopram and prolactin have been described in an earlier publication (Smith et al., 2002a, b). The prolactin assay is linear from 2.0 to 180 ng/ml, which is within the range of values obtained. Intra-assay precision of 16 replicates ranged from 7.8% to 8.2% with inter-assay precision of 16 replicates of 6.7–10.4%. The inter-assay coefficient of variation for citalopram is (2.9% at 15 ng/ml and 1.8% at 220 ng/ml, which is also within the range of values obtained (Foglia et al., 1997).

The data for citalopram and prolactin concentrations were analysed as areas under the curve (AUC increase from baseline, calculated using standard trapezoidal methods). The values were expressed as AUCs to integrate the data within the time frame of the PET scans, and because the main effects of time and group in the statistical analyses for both the AUC and individual time points were non-significant. For the comparison of Alzheimer’s disease patients to controls, the drug levels and prolactin data were analysed using repeated measures analysis of variance with diagnosis (control/Alzheimer’s disease patient) as a between subject factor and condition (two levels: placebo/citalopram) as a within subject factor. For the galantamine comparison in the Alzheimer’s disease patients, the drug levels and prolactin data were analysed using repeated measures analysis of variance with drug (baseline/galantamine) as a between subject factor and condition (two levels: placebo/citalopram) as a within subject factor.

The PET scans were performed using a GE Advance Tomograph in the Center for Neurosciences, the Feinstein Institute for Medical Research (Smith et al., 2002a, b). The subjects underwent intravenous infusions of placebo (250 ml of saline) or citalopram (40 mg of the drug diluted in 250 ml saline) over 60 min on two consecutive days. The order of placebo-drug administration was not randomized. The study was conducted as ‘single blind’ in that subjects were informed that they will receive either citalopram or placebo prior to each of the scans 30 min after the end of the infusion of placebo/citalopram, 5 mCi of [18F]-fluorodeoxyglucose ([18F]-FDG) was injected as an intravenous bolus. During radiotracer uptake, subjects were maintained in a quiet, darkened room with eyes open and ears unoccluded. Subjects were positioned in the scanner. First, a 10-min transmission scan and a 5-min twodimensional emission scan were acquired for photon attenuation correction. Then, a threedimensional emission scan began at 35 min after radiotracer injection and lasted for 10 min.

Glucose metabolic rates were calculated (in ml/100 g/min) on a voxel-by-voxel basis (Takikawa et al., 1993; Smith et al., 2002a, b). PET data processing was performed on the quantitative glucose metabolism images using the statistical parametric mapping program (SPM99; Friston et al., 1995). The images were smoothed with an isotropic Gaussian kernel (FWHM 8 mm for all directions). The glucose metabolic rates were normalized by scaling to a common mean value across all scans, after establishing that the global means did not differ significantly across groups and conditions (P > 0.05). For the comparison of the seven controls to the seven Alzheimer’s disease patients, differences in response (placebo/citalopram) between-groups (controls and Alzheimer’s disease patients) were compared using the multigroup: conditions and covariates option in SPM99 (Aim 1). For the comparison of the PET scans sessions before and during galantamine in the Alzheimer’s disease patients, the primary statistical comparisons involved (i) comparing the two placebo scans to evaluate the galantamine effect; (ii) comparing the two citalopram scans to measure the difference before and during galantamine treatment and (iii) comparing differences in the cerebral metabolic response to citalopram (citalopram–placebo) for the two conditions (baseline/galantamine; Aim 2). The between and within group comparisons were considered significant at a t-threshold >3.51 (z > 2.98, P < 0.001; uncorrected for multiple independent comparisons).

### Results

#### Clinical and cognitive data

The effects of galantamine on cognition (MMSE, ADAS-COG) and behaviour (NPI) and overall clinical improvement (CBIC plus) are shown in Table 1. The effect of time for the MMSE (F = 1.10, df = 2.12, P > 0.1), ADAS-COG (F = 1.5, df = 2.12, P > 0.1) and NPI (F = 0.18, df = 2.12, P > 0.1) was not significant. The effect of time for the CBIC plus was significant (F = 13.7, df = 2.12,
were as follows: Controls: placebo infusion: 1012/C6

Citalopram and prolactin concentrations

Comparison of Alzheimer’s disease patients to controls (Aim 1)

For the citalopram concentrations, the AUC was 6167 ± 819 for the controls and 5003 ± 1379 for the Alzheimer’s disease patients. While the concentrations were higher in the controls, the difference between the groups was not statistically significant ($F = 3.68$, $df = 1.13$, $P > 0.05$). For the prolactin concentrations, the AUCs were as follows: Controls: placebo infusion: 1012 ± 318 and citalopram infusion 2718 ± 1885; Alzheimer’s disease patients: placebo infusion: 1948 ± 1663 and citalopram infusion 2230 ± 1673. The effect of condition was significant ($F = 3.7$, $df = 1$, $P > 0.05$), but the effect of diagnosis ($F = 0.1$, $df = 1.12$, $P > 0.1$) and the condition by diagnosis interaction was not statistically significant ($F = 0.05$, $df = 1.12$, $P > 0.1$), even after including citalopram concentration as a covariate in the analysis ($P > 0.1$). For the individual data points for the citalopram and prolactin measures, repeated measures analysis of variance showed that the main effect of diagnosis and condition and the diagnosis by time by condition interactions were not significant ($P > 0.05$).

Galantamine Comparison in the Alzheimer’s disease patients (Aim 2)

For the citalopram concentrations, the AUC was 5003 ± 1379 and 4945 ± 1915 for the pre-treatment and galantamine treatment conditions and did not differ significantly ($F = 0.01$, $df = 1.6$, $P > 0.1$). For the prolactin concentrations, the AUCs were as follows: Pre-treatment: placebo infusion: 1948 ± 1663 and citalopram infusion 2230 ± 1673; during galantamine treatment: placebo infusion: 1150 ± 188 and citalopram infusion 2178 ± 1941. The effects of drug ($F = 0.27$, $df = 1.6$, $P > 0.1$) and condition ($F = 3.46$, $df = 2.19$, $P > 0.1$) and the drug by condition ($F = 1.10$, $df = 2.19$, $P > 0.1$) interaction were not significant.

Glucose metabolism PET data

The results of the SPM analysis of the cerebral metabolic data are shown in Tables 2 and 3.

Comparison of Alzheimer’s disease patients to controls (Aim 1)

The baseline comparison of resting cerebral glucose metabolism in the Alzheimer’s disease patients relative to controls (data not shown) demonstrated significant metabolic reductions in right middle temporal, left posterior cingulate and parietal cortices (precuneus and inferior parietal lobe), consistent with the pattern of metabolic deficits in Alzheimer’s disease shown in prior studies using voxel-based data analysis methods (Minoshima et al., 1997; Reiman et al., 2005). The cerebral metabolic effects of citalopram in the controls (citalopram–placebo conditions) are shown in Table 2, Panel A. The cerebral metabolic effects of citalopram in the Alzheimer’s disease patients (citalopram–placebo conditions) are shown in Table 3, Panel A. The comparison of the metabolic response to citalopram in Alzheimer’s disease patients relative to controls (difference between citalopram–placebo conditions between groups) are shown in Table 2, Panel B. The cerebral metabolic effects of citalopram prior to galantamine treatment are described in the previous paragraph (and shown in Table 3, Panel A).

Galantamine Comparison in the Alzheimer’s disease patients (Aim 2)

The cerebral metabolic response to citalopram during galantamine treatment (citalopram–placebo conditions) is shown in Table 3, Panel B. The effect of galantamine treatment on cerebral glucose metabolism in Alzheimer’s disease (comparison of placebo conditions is shown in Table 3, Panel C. The comparison of citalopram conditions in Alzheimer’s disease (during–prior to galantamine treatment) is shown in Table 3, Panel D. The areas of cerebral metabolic increased superimposed on an MR template are shown in Fig. 1.

Discussion

The results of the present study demonstrate a greater cerebral metabolic response to acute citalopram administration in Alzheimer’s disease patients relative to controls (Aim 1). The citalopram concentrations were non-significantly higher in the controls than patients. While the baseline prolactin concentrations were higher in the Alzheimer’s disease patients than the controls, the magnitude of increase in prolactin concentrations did not differ significantly between groups (even when covarying for the citalopram concentrations). The greater cerebral metabolic response to citalopram in the Alzheimer’s disease patients, despite the lack of change in plasma prolactin concentrations, may represent a
compensatory process for the reduced serotonin transporter, 5-HT2A and 5-HT1A receptor densities observed in Alzheimer’s disease patients shown by both neuropathological and neuroimaging methods (as reviewed by Meltzer et al., 1998) or to compensate for cholinergic dysfunction (Quirion et al., 1985; Quirion and Richard, 1987). Studies in the rat have shown compensatory changes in the serotonin system secondary to lesions of the basal forebrain cholinergic nuclei (Quirion et al., 1985, Quirion and Richard, 1987). Future studies should be undertaken to evaluate the mechanisms underlying the greater cerebral metabolic response to citalopram in Alzheimer’s disease patients relative to controls with respect to serotonergic and cholinergic mechanisms and the relationship to cognitive deficits and vulnerability to behavioural symptoms.

With respect to the clinical effects of galantamine, there was evidence of relatively consistent cognitive performance over the 8 months of the study and global clinical improvement as evidenced by the CBIC plus during the course of galantamine treatment. The plasma citalopram concentrations and the prolactin response to citalopram were not significantly different between baseline and galantamine conditions, which indicate that the cerebral metabolic effects of citalopram before and during galantamine treatment were not attributable to differences in citalopram concentrations. In both the comparison of Alzheimer’s disease patients to controls and of Alzheimer’s disease patients before and during galantamine treatment, the difference in the cerebral metabolic response was greater than the neuroendocrine prolactin response.

It is important to note that there are similarities between the functional neuroanatomic effects of acute citalopram and the pattern observed with behavioural activation paradigms, including mood induction, attention and memory tasks and during conditions of hunger and satiety (as reviewed in Fletcher et al., 1995; Tataranni et al., 1999; Liotti et al., 2001; Raichle et al., 2002a, b). These are all behaviours for which a modulatory role of serotonin has been described (as reviewed by Lucki, 1998). The regions modulated by acute citalopram include the heteromodal association cortices that in part, comprise the default network that is relatively activated even when the brain is not performing a task (Mazoyer et al., 2001; Raichle et al., 2001). While acute citalopram administration does not produce a unique pattern of neuroanatomic alterations, rather a brain network is activated that is involved in many affective, cognitive and motivational functions.

Table 2 The cerebral metabolic response to citalopram in Alzheimer’s disease and normal controls

<table>
<thead>
<tr>
<th>Talairach coordinates (x, y, z; mm)</th>
<th>Region</th>
<th>z-score</th>
<th>Cluster size K&lt;sub&gt;ε&lt;/sub&gt;a</th>
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</thead>
<tbody>
<tr>
<td><strong>Panel A. Normal controls: change in cerebral glucose metabolism between acute citalopram administration and placebo conditions</strong></td>
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<tr>
<td>Decrease in metabolism</td>
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<tr>
<td>12 -22 40</td>
<td>Right cingulate gyrus</td>
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<tr>
<td>-16 48 20</td>
<td>Left superior frontal gyrus</td>
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<td>237</td>
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<tr>
<td>10 -24 48</td>
<td>Right paracentral lobule (BA 06)</td>
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<td>6 28 34</td>
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<td>Left inferior parietal lobule (BA 40)</td>
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<td><strong>Panel B. Comparison of response in Alzheimer’s disease patients to controls</strong></td>
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<tr>
<td>Greater decreases in Alzheimer’s disease patients relative to controls</td>
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<tr>
<td>42 20 52</td>
<td>Right superior frontal gyrus (BA 08)</td>
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<td>Greater increases in Alzheimer’s disease patients relative to controls</td>
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<tr>
<td>-30 -70 -24</td>
<td>Left cerebellum</td>
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All coordinates are significantly different at the voxel level (P < 0.001 uncorrected).

a voxel = 8 mm<sup>3</sup>. BA = Brodmann Area.
The results of the cerebral metabolism data indicate that the nature of the acute cerebral metabolic effects of citalopram is different during treatment with galantamine compared with the response prior to treatment (Aim 2). As described, prior to treatment with galantamine, acute citalopram administration decreased cerebral metabolism in patients to a greater extent than controls. During galantamine treatment, citalopram increased metabolism in right anterior cortical regions (frontal and temporal cortices). Galantamine treatment was associated with an increase in metabolism in right posterior (parietal and occipital association) cortical regions compared to baseline. When considering the galantamine effect (citalopram–placebo conditions during galantamine treatment), significant increases in metabolism was observed in right frontal and parietal cortices. When comparing the two citalopram conditions (citalopram during–prior to galantamine treatment), significant increases in metabolism were observed in frontal cortices.

<table>
<thead>
<tr>
<th>Panel A. The cerebral metabolic response to citalopram prior to galantamine treatment (citalopram-placebo conditions)</th>
<th>Decrease in metabolism</th>
<th>Right superior frontal gyrus (BA 10)</th>
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<td></td>
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<td>Right cerebellum</td>
<td>3.49</td>
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<tr>
<th>Panel C. The effects of galantamine treatment on cerebral glucose metabolism (comparison of placebo conditions)</th>
<th>Decrease in metabolism</th>
<th>Right inferior frontal gyrus</th>
<th>4.39</th>
<th>335</th>
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<tr>
<td></td>
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<td>Right pre-central gyrus</td>
<td>3.37</td>
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<td>Increase in metabolism</td>
<td></td>
<td>Left inferior frontal gyrus</td>
<td>3.04</td>
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<td>Right precuneus</td>
<td>4.37</td>
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<td>Right inferior parietal lobule (BA 40)</td>
<td>3.19</td>
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<td></td>
<td>Right middle occipital gyrus (BA 19)</td>
<td>3.32</td>
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<th>Panel D. Comparison of citalopram conditions (during–prior to galantamine treatment)</th>
<th>Decrease in metabolism</th>
<th>Left middle temporal gyrus</th>
<th>4.42</th>
<th>4218</th>
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<tr>
<td></td>
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<td>Left putamen</td>
<td>4.14</td>
<td>4218</td>
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<tr>
<td></td>
<td></td>
<td>Left cerebellum</td>
<td>3.18</td>
<td>177</td>
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| Increase in metabolism | | Right middle frontal gyrus (BA 08) | 2.98 | 638 |
| | | Left middle frontal gyrus | 3.39 | 638 |
| | | Right paracentral lobule (BA 06) | 3.26 | 667 |
| | | Right posterior cingulate gyrus (BA 31) | 3.07 | 667 |
| | | Right fusiform gyrus | 3.74 | 6096 |

All coordinates are significantly different at the voxel level (P<0.001 uncorrected).

a voxel = 8 mm³.

BA = Brodmann Area.
addition, a glutamatergic mechanism may be involved in the cere-
mate is the primary neurotransmitter (Fagg and Foster, 1983). In 
tations observed are in cortico–cortico pathways for which gluta-
be involved. In the present study, the cerebral metabolic altera-
tions observed are in cortico–cortico pathways for which gluta-
neuraltic activity represents the final common pathway of neurochem-
afflicted both the prolactin and metabolic data. As glucose meta-
icholinergic–serotonergic interventions. This region is particularly 
region is particularly 
A synergistic effect of the two medications.
lopram administration during galantamine treatment is not only a 
function of the effect of galantamine on metabolism, but may be 
a synergistic effect of the two medications.

The cerebral metabolic effects of galantamine observed in the 
study are similar to the metabolic effects reported in pre-
vious studies of galantamine and other cholinesterase inhibitors 
(Tune et al., 2003; Mega et al., 2005). Compared to the present 
study, previous studies have not shown an increase in metabolism 
in the posterior cingulate gyrus as was observed with combined 
cholinergic–serotonergic interventions. This region is particularly 
important as the posterior cingulate has been the site of the earliest 
metabolic reductions in Alzheimer’s disease (Minoshima et al., 
1997; Reiman et al., 2005). With respect to the neurochemical 
mechanisms underlying the present findings, it is important to 
note that the results obtained with galantamine may be attribut-
abe to either the cholinesterase inhibition or nicotinic receptor 
modulation mechanisms of the drug or both, in addition to sec-
ondary neurochemical effects on other neurotransmitter systems 
such as dopamine (Harvey et al., 1995), which would have 
affect an the prolin and metabolic data. As glucose meta-
bolic activity represents the final common pathway of neurochem-
ical activity in the brain, the data represent the functional 
neuroanatomic alterations associated with the pharmacologic 
interventions. The evaluation of the regional pattern of change 
may suggests which neurochemical pathways mechanisms may 
be involved. In the present study, the cerebral metabolic altera-
tions observed are in cortico–cortico pathways for which gluta-
mate is the primary neurotransmitter (Fagg and Foster, 1983). In 
addition, a glutamatergic mechanism may be involved in the cere-
bral metabolic deficits observed in Alzheimer’s disease (Smith 
et al., 1992). As both citalopram and galantamine have been 
shown to affect glutamate neurotransmission, consistent with a 
modulatory role of serotonin and acetylcholine (Golembiowska 
and Dziubina, 2000; Takada-Takatori et al., 2006), the metabolic 
alterations observed may represent a secondary, synergistic effect 
of cholinergic and serotonergic agents on the glutamate system. 
Several limitations of the present study should be considered in 
interpreting the results. In the study design, a group of Alzheimer’s 
disease patients scanned on two occasions without cholinesterase 
inhibitor treatment was not included. Given the long duration of 
treatment, it would not have been feasible to identify Alzheimer’s 
disease patients who were not willing to take cholinesterase inhib-
itors over the time interval of study. Second, as described in the 
Materials and Methods Section, the Alzheimer’s disease patients 
did show evidence of behavioural symptoms as assessed by the 
NPI (e.g. apathy, depression, anxiety, agitation), but did not meet 
criteria for an axis I DSM IV psychiatric diagnosis. It is possible 
that the behavioural symptoms may have introduced variability in the 
glucose metabolism data as has been shown in previous studies 
(Lopez et al., 2001a, b), as well as in the response to serotonergic 
or cholinergic agents. Given the natural history of Alzheimer’s 
disease, it would be difficult to identify subjects free of neuropsy-
chiatric symptoms. Thirdly, the issue of the variability of the data 
and the small sample size should be considered. The sample size is 
small because only patients who had not been treated previously 
with cholinesterase inhibitors were enrolled and such patients are 
difficult to identify as the majority of patients are treated. The 
pattern of results and direction of the effects are consistent with 
the study hypothesis based on previous glucose metabolism stud-
ies with acute citalopram and cholinesterase inhibitors (sepa-
ately) and with preclinical data. With respect to the citalopram 
and prolactin concentrations, the standard deviations for the mea-
surements are large, but are not explained by intra-assay variabil-
ity as discussed in the Materials and methods section. In reviewing 
the data for the individual time points (as shown in the 
Supplementary material), the variability lies in the magnitude of the 
peak effects. For the time points other than the peak citalo-
pram concentrations and prolactin effect, the variability observed 
is consistent with the precision of the measurements. Thus, during 
the time of maximal increase in citalopram and prolactin concen-
trations, the magnitude of response was variable across subjects. 
With respect to the cerebral metabolism data, the data for the 
significant voxels were evaluated to determine the degree of 
between subject variability in response. Representative plots for 
the posterior cingulate gyrus for two of the contrasts (placebo 
versus citalopram prior to treatment and citalopram during treat-
ment versus pre-treatment) are provided in the Supplementary 
material. In both cases, the direction of the effects between sub-
jects is relatively consistent, although the magnitude change differs 
between subjects. Thus, even though the sample size is small, the 
direction of the metabolic effects is relatively consistent. Using 
the same acute citalopram paradigm in geriatric depressed patients 
with a similar sample size, the results of a study of six patients 
and the results of a larger group of 16 patients were similar 
(Smith et al., 2002a, b, 2008). Thus, despite the small sample 
size, the findings are comparable to or less variable, given the 
variability typically observed in neuroimaging studies.
The significance of acute citalopram administration relative to the clinical use of citalopram on a chronic basis should be considered. Acute citalopram administration is intended as a ‘challenge’ to measure the consequences of an acute increase in serotonin concentrations on cerebral metabolism. Acute citalopram administration (as in the present study) is associated with 70% serotonin transporter occupancy, which is comparable with the magnitude of blockade observed with chronic citalopram treatment (Hinz et al., 2008). While there is a neurochemical effect of acute citalopram with respect to occupancy of the initial site of action and increases in extracellular serotonin (Kreiss et al., 1993), the clinical antidepressant effect in depressed patients is not observed until after chronic administration. In comparing the chronic to acute effects of citalopram in geriatric depressed patients, similar changes in metabolism are observed, in addition to alterations in other regions (e.g. putamen, parahippocampal gyrus and amygdala) and the effects tend to be bilateral rather than unilateral (Smith et al., 2002a, b). Studies in geriatric depressed patients have shown that greater magnitude of acute cerebral metabolic response to citalopram is associated with greater improvement of depressive symptoms after a 12-week clinical trial of citalopram (Smith et al., 2008). Thus, these data suggest that acute cerebral metabolic response to citalopram may reflect the capacity of the brain to respond to chronic antidepressant treatment. With respect to the present study, the cerebral metabolic response to acute citalopram in Alzheimer’s disease is greater in patients than controls. This metabolic response may represent a compensatory process secondary to the substantial loss of serotonin transporter and receptors in Alzheimer’s disease. As the brain demonstrates a capacity to respond to acute citalopram in Alzheimer’s disease, this may suggest why there is a synergic metabolic effect of combined interventions. Similar to the depression data, the capacity of the brain to respond may be related to clinical and metabolic outcomes. This may also suggest why chronic serotonergic interventions have been effective in Alzheimer’s disease and why combined chronic, serotonergic–cholinergic treatments may be an effective therapeutic strategy. Such a study of chronic treatment with both agents, would be a logical next step for the research.

In contrast to studies of serotonin transporters and receptors in Alzheimer’s disease, the present study provides evidence for an altered dynamic response of the brain to an acute pharmacologic increase in serotonin, as well as a synergistic effect of serotonergic and cholinergic interventions, consistent with preclinical studies of interactions between the two neurotransmitter systems. The present study provides preliminary support for a synergistic effect of acute SSRI administration and cholinesterase inhibitor treatment on cerebral metabolism in Alzheimer’s disease. Several lines of preclinical and clinical evidence support further investigations of the neurobiological effects of combined chronic treatment with the two classes of agents. With respect to the SSRI’s, the evidence of a neurotrophic effect of SSRI’s, as well as recent evidence of a prophylactic effect of SSRI’s in animal models of Alzheimer’s disease had led to the suggestion that chronic use of the SSRI in neurodegenerative disease may improve function and slow disease progression (Duman, 1998, Nelson et al., 2007). Thus, data in animals suggest that a greater clinical benefit may be achieved by combined treatment. With respect to clinical studies, the results of several double blind, placebo controlled studies indicate that addition of an SSRI to a cholinesterase inhibitor produced greater functional improvement in Alzheimer’s disease patients compared with cholinesterase inhibitor treatment alone (Finkel et al., 2004). SSRIs have been shown to be effective in treating depression secondary to Alzheimer’s disease, which may also improve function (Lyketsos et al., 2003). The observations from clinical studies of combined SSRI and cholinesterase inhibitor treatment, considered with the preliminary neuroimaging data suggest that future studies should be undertaken to further evaluate the cognitive and neurobiological consequences of chronic, combined cholinergic and serotonin interventions in Alzheimer’s disease.

Supplementary material
Supplementary material is available at Brain online.

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