

Antemortem measurements of neurotransmission: possible implications for pharmacotherapy of Alzheimer's disease and depression

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Abstract

Aspartic acid, 5-hydroxyindoleacetic acid, glutamic acid, homovanillic acid and 3-methoxy-4-hydroxyphenylethylene glycol was determined in samples of ventricular fluid from 82 subjects. Laminar distribution of the total number (B_{max} value) of serotonin 1A receptors was determined on seven neurosurgical samples of neocortex. Apart from an association in a small subgroup of subjects between homovanillate concentration and corticosteroid medication, no complicating influences of treatment preceding operation were found. The content of the serotonin metabolite alone was significantly reduced in intractable depressive illness (bipolar and major depressive disorders) compared with neurological conditions subdivided into Alzheimer's disease, other dementias and other conditions. There was no other significant difference between these groups for the compounds measured. The total number of serotonin 1A receptors was highest in the superficial layers, being considerably higher than in the rat, irrespective of cortical layer. This part of the study indicated that these receptors are important for regulating activity of human corticocortical glutamatergic neurons. The results are discussed in relation to treating depression with serotonergic agents and targeting corticocortical glutamatergic neurons as well as acetylcholine in Alzheimer's disease.

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Development of pharmacotherapy for Alzheimer's disease with agents that would aim to improve, if not restore, neurotransmission has been viewed with pessimism.¹⁻³ The disease is a slowly progressing disorder, yet such conclusions were based on biochemical analyses of postmortem tissue, often from selected severely affected subjects at the end-point of the disease process, that indicated multiple transmitter deficits had occurred. Similarly, it could be concluded that complex transmitter related changes were also a feature of depressive illness as a myriad of antidepressant drugs, with a host of proposed transmitter actions, have recently emerged.⁴ A few studies have provided information about neurotransmission in living patients at a more central level than may be obtained by studying lumbar cere-

brospinal fluid. For instance, the investigation of ventricular fluid showed reduced homovanillate concentration in patients with Parkinson's disease.⁵ Analysis of cortical tissue removed at neurosurgical craniotomy is another approach and has included the study of transmitter receptors.^{6,7} Results reported here extend these approaches to provide new information about catecholamine, excitatory dicarboxylic amino acid and serotonin transmission of human brain, in particular with respect to Alzheimer's disease and depressive illness.

Methods

Samples of ventricular cerebrospinal fluid were obtained from 52 patients who had intractable depression. Electroconvulsive therapy (ECT), as well as high dose and combination drug treatment had not been effective during the period of presurgical assessment and so they underwent the psychosurgical operation of stereotactic subcaudate tractotomy.⁸ All patients had fulfilled the requirements of the Mental Health Act, 1983. Details of current and previous treatments were recorded. Drugs were classified into antidepressants, antipsychotics, tranquillisers, lithium salts, and other psychotropic drugs; patients suffered with affective disorders and were diagnosed according to the research diagnostic criteria.^{9,10} Forty six patients had either major depressive disorder or bipolar disorder and six had other psychiatric disorders (four with obsessive compulsive disorder and two with phobic disorder). Patients with major depressive disorder were subclassified as unipolar psychotic or unipolar simple according to presence or absence, respectively, of delusions or hallucinations, or both.⁹ The unipolar psychotic group had three men and five women with a mean age of 60 (46-74) years. The unipolar simple group had twelve men and ten women with a mean age of 48 (21-68) years. The bipolar disorder group had two men and fourteen women with a mean age of 47 (29-68) years.

The second group, with obsessive compulsive and phobic disorders, had four men and two women with a mean age of 42 (27-55) years. During the course of the operation, air encephalography was performed to identify the precise location of the lesion and cerebrospinal fluid was removed from the lateral ventricle of necessity. Samples of CSF were maintained at minus 70°C until the contents of free amino acids (aspartic acid and glutamic acid)

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and monoamine transmitter metabolites (5-hydroxyindoleacetic acid, 5-HIAA; homovanillic acid and 3-methoxy-4-hydroxyphenylethylene glycol, MHPG) were determined (blind to patient characteristics) by high performance liquid chromatography and fluorescence detection following derivatisation with an *o*-phthalaldehyde reagent for amino acids¹¹ and electrochemical detection for monoamines.¹² An anomalous glutamate value (10.8 nmol/ml) was excluded from the analysis.

Cerebrospinal fluid was also obtained from the lateral ventricles of adult neurological patients including samples from procedures for insertion of ventriculoperitoneal shunts and craniotomy for brain tumours. This group had six men and six women with a mean age of 48 (21–69) years. Diagnoses were normal pressure hydrocephalus (three), aqueduct stenosis (three), craniopharyngioma (two), posterior communicating artery aneurysm, malignant glioma, meningioma and epidermoid tumour (one each). Shunts were not inserted into the patient with meningioma and one patient with craniopharyngioma. In those shunted, two patients were using carbamazepine, one in combination with diazepam and the other with corticosteroids. In the craniotomy subgroup, there were two patients using antiepileptics and corticosteroids. The other neurological samples of ventricular fluid were obtained from demented patients at diagnostic craniotomy of the superior frontal lobe.¹³ A group with Alzheimer's disease histology had six men and three women with a mean age of 60 (54–66) years. The remainder had no specific histological changes, five men and two women with a mean age of 54 (50–66) years. The demented patients were not receiving any drugs during at least the week preceding operation. There were no significant differences in age between the groups except for the patients with Alzheimer's disease, who were older ($p < 0.05$) than the other groups.

The investigated compounds were detectable in all samples assayed except for glutamate in 10 subjects, where there were contaminating peaks.

During the psychosurgical operation, a small piece of cerebral cortex was always removed bilaterally from the frontal lobes, to make insertion of a cannula as safe as possible. Cryostat sections were prepared from two antidepressant free¹⁰ and two antidepressant treated¹⁰ patients with major depressive and bipolar disorders, three age-matched controls (whose frontal cortex appeared normal when removed at neurosurgery for access to deep tumours) and rats of the Sprague-Dawley strain. Sections were incubated with eight concentrations (0.5–5 Tnmol/l) of [³H]-8-hydroxy-2(n-dipropylamino)tetralin, the prototypical serotonin 1A partial agonist, with quantitation using computerised receptor autoradiography (Quantimet 570, Leica, Cambridge).^{14,15}

Results were expressed as mean (SE). Variances of group means were compared using Fisher's test of exact probability and, if there

was no significant difference, one way analysis of variance (ANOVA). The least significant difference test, if appropriate, or the two-tailed Student's *t* test were used to compare group means. Where there was a significant difference, Kruskal-Wallis, ANOVA, if appropriate, and the Mann-Whitney U-test or Wilcoxon 2 sample test were used to compare group means.

Results

Measured compound concentrations in the neurological patients were not influenced by drug treatment, operation type, age, sex or response to operation (data not shown) except for homovanillate which was higher ($p < 0.01$) in corticosteroid-treated subjects. For the patients with affective disorders, measured compound concentrations were not influenced by age or sex, except for homovanillate content with age (Pearson's correlation $r = -0.40$, $n = 38$, $p < 0.02$). Measured compound concentrations were not affected by drug treatment (antipsychotics or antidepressants, including lithium) or ECT, based on treatment during the six months (or 12 months for ECT) preceding collection of sample (data not shown). Most patients studied (31) were receiving drugs from two categories or less. There were no differences in the concentrations of investigated compounds between this group and the group of 21 subjects receiving polypharmacy, that is, drugs from three or more categories (data not shown). For those without polypharmacy there was no effect of drug treatment or ECT on the monoamine metabolites or amino acids (data not shown).

The figure shows that the major serotonin metabolite (5-HIAA), aspartate and glutamate values were distributed over relatively wide ranges, except for aspartate in the neurological subjects. When these values for the various groups were compared, no significant differences were found in aspartate and glutamate concentrations. This was also the case for metabolites of catecholamine transmitters (homovanillate and MHPG: table 1). Whereas the 5-HIAA value was significantly lower in the depressed group (DEP, fig), compared with neurological groups, the concentration of 5-HIAA and those of the other investigated compounds did not significantly differ between subgroups (data not shown).

5-HIAA content was not significantly correlated with aspartate or glutamate values for any group of comparable numbers (data not shown), except for the demented subjects where aspartate and 5-HIAA concentrations were negatively correlated (Pearson's correlation $r = -0.63$, $n = 15$, $p = 0.01$).

Within the human neocortex there were more serotonin 1A receptors in superficial layers than in either deep or superficial layers of the rat (table 2). In the human, binding was highest in superficial layers and by comparison with adjacent histological sections (not shown) it was established that this region of the autoradiograph corresponded mainly to cortical layers I and II.¹⁶ Binding parameters

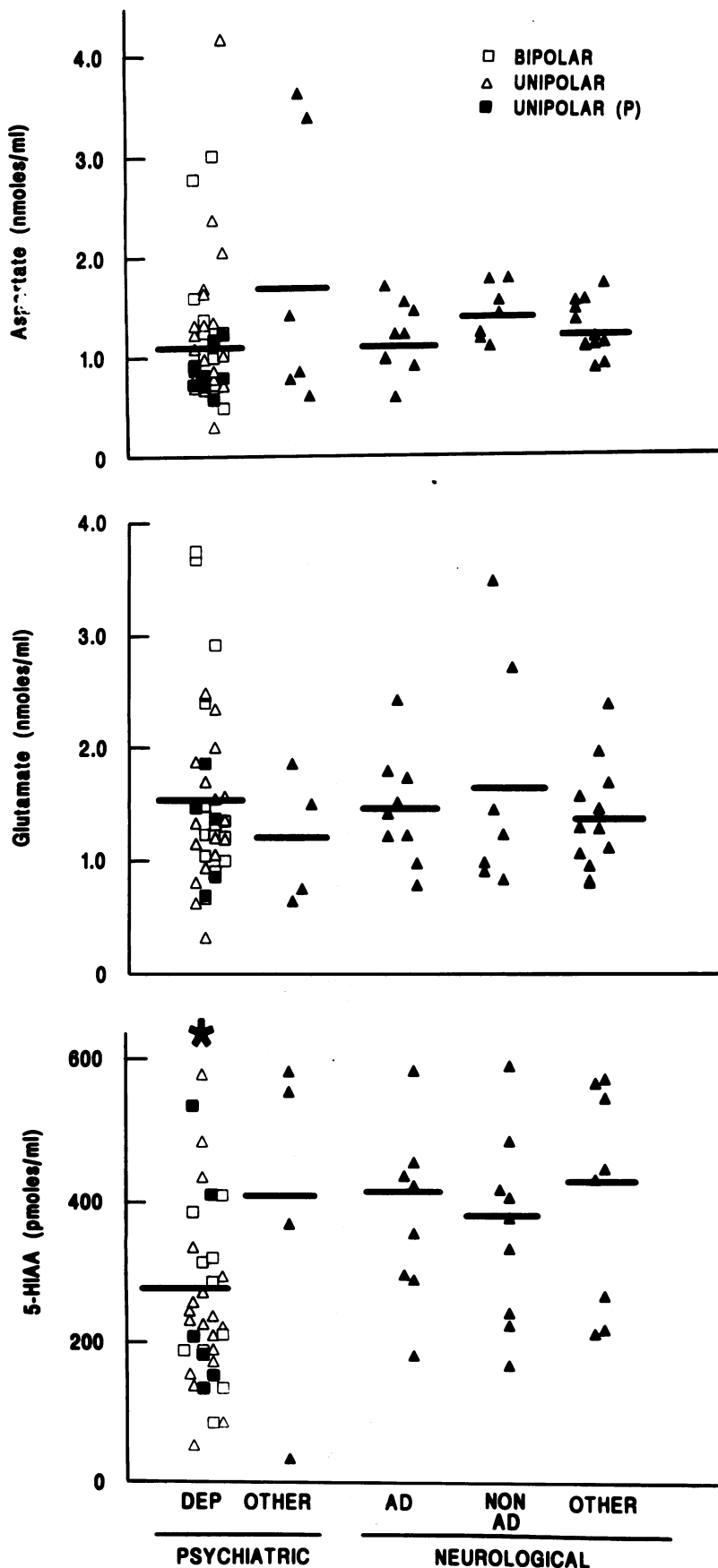


Figure 1 Excitatory dicarboxylic amino acids and the major serotonin metabolite 5-HIAA in ventricular fluid. Concentrations for individual patients and means (horizontal lines) are shown for five groups of patients: DEP (bipolar or major depressive disorders, subclassified as discussed in the text); Other (other psychiatric disorders); AD (Alzheimer's disease); Non-AD (no specific histological changes); and Other (other neurological patients). The asterisk identifies the only significant difference ($p < 0.05$) between groups (ANOVA and the least significant difference test; groups consisting of only four patients were excluded from the analysis).

appeared independent of medication (data not shown; see also¹⁷) and were lower in the DEP samples compared with controls, in superficial layers only (table 2).

Discussion

Laminar distribution of binding to the serotonin 1A receptor was different in postmortem human tissue compared with rapidly processed rat brain,¹⁸⁻²⁰ so it was important to eliminate the possibility that the difference was a post-mortem artefact. To our knowledge this is the first study to employ rapidly processed human tissue and is also new, for both humans and a laboratory animal, in the use of a selective [³H]ligand to determine kinetic parameters for the receptor in superficial and deep cortical layers. In the rat the receptor was enriched (by comparison with all other receptors studied,^{15,21}) on corticofugal pyramidal neurons,^{14,15} which almost certainly released either aspartate or glutamate as transmitter.²² Human neocortical pyramidal neurons studied electrophysiologically using intracellular recordings,⁶ responded to the prototypical serotonin 1A partial agonist used here. In Alzheimer's disease, the overall B_{max} value for the receptor was reduced in the neocortex, where pyramidal neuronal loss had occurred.²³ A role for these cells in depression has been proposed^{9,24} and this was consistent with the low B_{max} value in table 2 for depression. In human brain the B_{max} value was highest in superficial layers (table 2), corresponding to cortical layers I and II (see above). These observations provide evidence that, in humans, the receptor is predominantly a component of ipsilateral projecting²⁵ corticocortical pyramidal neurons. For deep and superficial cortical layers of the rat brain, the B_{max} value was lower than for human superficial layers (table 2), yet it is well known that neuronal density is higher in the rat. This suggests that humans have more serotonin 1A receptors per corticocortical neuron and that the receptors are important for regulating activity of the cells, which probably release glutamate.²⁶ Thus it should be possible to discover drugs to target this subpopulation of cortical neurons.

Apart from the biochemical analyses, histological investigation of Alzheimer's disease has also focused on the end-point stage, and it has been emphasised that interconnections between many parts of the cortex are disrupted at multiple levels.³ Even if new lines of research (as reviewed²⁷) eventually yield treatment to slow progression of pathology, drugs that affect neurotransmission will still be needed in most patients to improve functional activities that are already impaired. The present data indicate relative sparing of dopamine, noradrenaline and serotonin (table 1, fig), as has already been found for somatostatin.¹³ This is consistent with most results for the transmitter biochemistry of cortical biopsy samples, and includes γ -aminobutyric acid (as reviewed²⁶). Although the present results do not indicate any abnormality of ventricular 5-HIAA concentration, 5-HIAA and aspartate content may

Table 1 Concentrations of catecholamine metabolites, mean (SE), in ventricular fluid

Patient groups	No of patients	Homovanillate (pmol/ml)	MHPG (pmol/ml)
Psychiatric			
DEP	34-35	1444 (106)	45.1 (4.5)
Other	4	2436 (583)	50.1 (8.8)
Neurological			
AD	7-8	1190 (159)	32.4 (4.9)
Non-AD	9	1008 (122)	ND
Other	5-8	985 (49)	29.7 (4.7)

ND, not determined; DEP = bipolar or major depressive disorders; AD = Alzheimer's disease. Patient groups as defined in the text, except that homovanillate values of corticosteroid-treated patients with ventriculoperitoneal shunts and brain tumours were excluded. There were no significant differences (ANOVA, least significance difference test; groups comprising only four patients were excluded from the analysis).

Table 2 Binding parameters of the serotonin 1A receptor, mean (SE), in frontal neocortex of human (region of Brodmann area 9) and rat (Fr1/FR2) brain

Cortical layer	Human*		
	DEP (n = 4)	Control (n = 3)	Rat (n = 5)
Superficial			
B _{max} (fmol/mg tissue)	62.3 (2.9)†	87.5 (4.5)	28.3 (1.8)
K _d (nmol/l)	0.50 (0.09)‡	0.83 (0.09)	2.54 (0.38)
Deep			
B _{max} (fmol/mg tissue)	25.3 (1.3)	28.3 (5.8)	36.8 (3.4)
K _d (nmol/l)	0.93 (0.25)	1.03 (0.32)	1.75 (0.48)

DEP = bipolar or major depressive disorders.

* Mean B_{max} value for superficial layers of all samples (n = 7) was higher (p < 0.005, Student's *t* test) than for either deep or superficial layers of the rat.

† p < 0.01.

‡ p < 0.06, from control (Student's *t* test), deep layers showed no significant differences from control.

be abnormally related in demented patients. Even in the postmortem Alzheimer's diseased brain, however, half the many cortical areas assayed had no evidence of a selective reduction in presynaptic serotonergic activity. Indeed the surviving serotonergic nerve endings seem most active in the most demented patients (for reference see²⁸).

The lack of change in excitatory amino acids (fig) suggests that corticostriatal excitatory neurons (as reviewed²²) are not affected appreciably. This provides further evidence that Alzheimer's disease is characterised by two selective and critical deficits: circumscribed corticocortical glutamatergic²⁹ and cholinergic degeneration. Cortical pyramidal neurons are probably subject to cholinergic modulation^{6,21,30} so improved cholinomimetics³¹ should complement approaches aimed at slowing the progression of the pathology. Partial agonists of glutamate may also be useful.^{29,32} Selective serotonin 1A receptor antagonists^{33,34} should promote all effects of the remaining glutamate transmitter pool by inhibiting the tonic hyperpolarising action of endogenous serotonin on pyramidal neurons, thereby compensating for reduced glutamatergic transmission. It is well known that drugs showing serotonin 1A agonism reduce aggression and depression in animals so this strategy will require careful evaluation. Use of rats may be inadequate to predict clinical consequences of treatment with 1A drugs because the receptor in this species and humans clearly have different distributions (table 2).

Aspartate has been proposed as the major transmitter of the corticostriatal excitatory neurons.²² The lack of change in the concen-

tration of this amino acid in ventricular fluid from depressed patients indicates that these neurons are preserved. This is supported by the observation that the serotonin 1A receptor was also unchanged in the deep cortical layer (table 2). In cerebral cortex from a similar group of patients, aspartate concentration was increased,⁹ so the most straightforward interpretation for reduced 1A receptor numbers (table 2) and increased aspartate is that this amino acid is not enriched in corticocortical neurons.

The main finding of this study of ventricular fluid from over 80 subjects is that the group with either major depressive disorder or bipolar disorder had a mean 5-HIAA value that was significantly lower than neurological controls. No other difference between any of the diagnostic groups was observed for the measured compounds. This is the first study that provides evidence of selective deficit in serotonin transmission in living patients with depressive illness at a more central level than may be obtained with lumbar fluid. The conclusion was equivocal in previous studies of ventricular fluid that attempted to address this issue³⁵ and results on lumbar fluid were contradictory.³⁶ Now that it has been possible to establish an indication for a specific medication on the basis of biochemical criteria,⁴ it is hoped that the heterocyclic antidepressants, which have unpleasant effects,³⁷ will be supplanted by the modern serotonin uptake blockers.³⁷ Compounds active at serotonin 1A receptors are under investigation (for example, partial agonist drugs,³⁸ not previously considered⁴), but the clinical consequences of activation and inhibition of serotonin 1A receptors need to be elucidated.³⁴

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