British Journal of Psychiatry (1992), **160**, 638–642

Effect of Psychotropic Drugs on Excitatory Amino Acids in Patients Undergoing Psychosurgery for Depression

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Samples of ventricular CSF were taken from 52 consecutive patients admitted for psychosurgery for intractable depression. Concentrations of asparagine, aspartate, glutamine, glutamic acid, and serine were determined. Glutamate and aspartate concentrations, implicated in excitotoxic brain damage, were not affected by various types of psychotropic drug treatment. Serine, a modulator of glutamate responses, was significantly elevated in samples from subjects receiving antidepressants. These subjects responded poorly to the operation. Psychotropic drugs are unlikely to be neurotoxic. Nevertheless, antidepressants may influence excitatory neurotransmission.

Research into the biological basis of depression and schizophrenia has recently focused on changes in the cerebral cortex (Procter & Bowen, 1987; Jeste *et al*, 1988; Kerwin, 1989), and on the pyramidal neurons in particular (Deakin *et al*, 1989; Francis *et al*, 1989). This is surprising, as the neurotransmitters previously implicated in these conditions are those of subcortical perikarya (e.g. serotonin and dopamine). A major transmitter of cortical neuronal signals is the excitatory amino acid glutamate. Under certain circumstances it is also neurotoxic, an effect mediated through three subtypes of specific receptors, particularly the N-methyl-D-aspartate subtype, where its actions are modulated by neutral amino acids such as glycine and serine (Johnson & Ascher, 1987; Reynolds & Miller, 1988; Procter *et al*, 1989). Endogenous excitotoxic mechanisms have been proposed to account for cortical damage, not only in conditions such as Alzheimer's disease where distinctive histopathology occurs together with abnormal glutamate neurotransmission in some areas (Bowen, 1990), but also in schizophrenia (Kerwin, 1989).

Glutamate-mediated excitotoxicity has not to date been implicated in the cortical abnormalities of affective disorders, but it has been suggested that the cortical changes in depressed as well as schizophrenic subjects are related to their treatment rather than the disorder itself (Wood *et al*, 1990). An alternative possibility is that the clinical effects of psychotropic drugs are in part mediated through changes in neurotransmission by excitatory amino acids. Although established sources (e.g. see section 4.3 of the *British National Formulary* (Joint Formulary Committee, 1990)) indicate that antidepressants can precipitate seizures, neuronal overactivity in rodents was attenuated by antidepressants (Clifford *et al*, 1985), possibly through direct action on the N-methyl-D-aspartate subtype of glutamate receptor (Reynolds *et al*, 1988; Trullas & Skolnick, 1990).

To investigate mechanisms by which psychotropic drugs may have their action, we have assayed 52 samples of ventricular cerebrospinal fluid (CSF) from depressed patients, and related therapy with various types of psychotropic drugs to the concentrations of several amino acids, including those with actions at the N-methyl-D-aspartate receptor.

Method

Samples of ventricular CSF were obtained from 52 patients with intractable depression admitted to the Geoffrey Knight Unit for Affective Disorders, Brook General Hospital, London. Electroconvulsive therapy (ECT), as well as high-dose and combination drug treatment (Hale *et al*, 1987), had not been effective during pre-surgical assessment at the unit, and so patients underwent the psychosurgical operation of stereotactic subcaudate tractotomy (Francis *et al*, 1989; Poynton *et al*, 1988). All patients had fulfilled the requirements of the Mental Health Act 1983.

Before the operation, all patients underwent detailed psychiatric and medical assessment, including the 17-item Hamilton Rating Scale for Depression (see Francis *et al*, 1989). Details of current and previous treatments were recorded. Drugs were classified into antidepressants, antipsychotics, tranquillisers, and lithium salts, as listed in sections 4.3, 4.2.1 and 4.2.2, 4.1, and 4.2.3, respectively, of the *British National Formulary* (Joint Formulary Committee, 1990), and other psychotropic drugs. All patients had affective disorders and were diagnosed according to the Research Diagnostic Criteria, as described (Francis *et al*, 1989). Assessment of post-operative outcome was made at one year, using our global outcome scale (Poynton *et al*, 1988).

During the operation, air encephalography is performed to permit the precise placement of the lesion, and ventricular

fluid is removed of necessity. Samples of this were maintained at −70°C until the contents of asparagine, aspartic acid, glutamine, glutamic acid and serine were determined (blind to patient characteristics) by high-performance liquid chromatography and fluorescence detection following derivatisation with an ophthaldialdehyde reagent (Procter *et al*, 1988). One anomalous glutamate value (10.8 nmol/ml) was excluded from the analysis.

With this method, in a control series of lumbar CSF (Procter *et al*, 1988), serine content (21.6 (1.4) nmol/ml, $n=15$) was within the established normal range for adults (Hagenfeldt *et al*, 1984; Crawford *et al*, 1988; Qureshi & Baig, 1988), as were values for the other amino acids in these samples (Procter *et al*, 1988).

Ventricular fluid was also obtained from adult neurological patients, and included samples from procedures for insertion of ventriculo-peritoneal shunts and for craniotomy for brain tumours. This group comprised six men and six women (mean age 48, range 21–69 years). Diagnoses were normal-pressure hydrocephalus (3), aqueduct stenosis (3), craniopharyngioma (2), posterior aneurysm, malignant glioma, meningioma, and epidermoid tumour (1 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 anti-epileptics and corticosteroids. Measured concentrations of amino acids were not influenced by drug treatment, operation type, age, sex, or response to operation (data not shown). The other neurological samples of ventricular fluid were obtained from demented patients at diagnostic craniotomy. A group with Alzheimer's disease confirmed by histology comprised six men and three women (mean age 60, range 54–66 years). The remainder of the demented patients (5 men, 2 women, mean age 54, range 50–66 years) had no specific histological changes.

Results are expressed as means (s.e.). Variances of group means were compared using the Fisher F test, and if there was no significant difference, one-way analysis of variance (ANOVA), if appropriate, and the two-tailed Student's t-test were used to compare group means. Where there was a significant difference, Kruskall–Wallis ANOVA, if appropriate, and the Mann–Whitney U test or Wilcoxon two-sample test were used to compare group means.

Results

The values for aspartate and glutamate for neurological samples of ventricular fluid (see Fig. 1 legend), were within the reported ranges (Engelsen *et al*, 1985; Perschak *et al*, 1987; Crawford *et al*, 1988). The serine values were higher than in lumbar fluid (see also McGale *et al*, 1977) but similar to that reported for lumbar fluid of the newborn (Spink *et al*, 1988).

Thirty-one of the patients with affective disorder were receiving drugs from two or fewer categories. There were no differences in the concentrations of investigated compounds between this group and the 21 subjects receiving drugs from three or more categories (data not shown). However, to minimise possible complicating influences, detailed analysis has been confined to those without polypharmacy. The investigated compounds were detectable in the ventricular CSF samples of all 31 patients except for glutamate in three subjects. There was no effect of antipsychotic medication on these compounds, with the exception of glutamine (476 (6) nmol/ml for those taking antipsychotics v. 489 (3) nmol/ml for those not, $P<0.05$, Student's t-test).

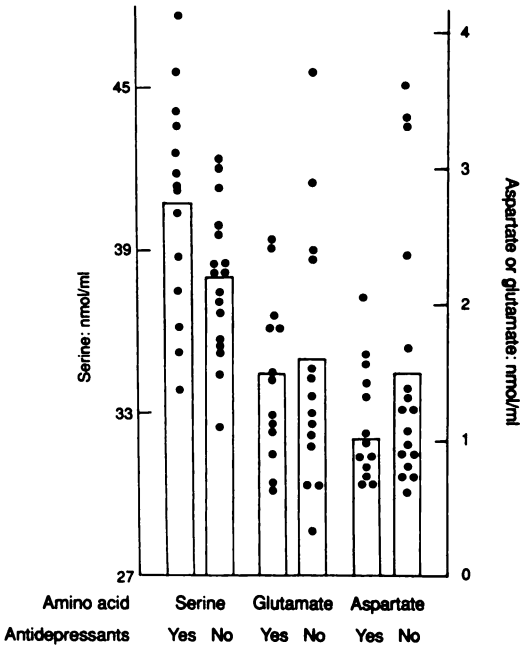

Fig. 1 Concentration in ventricular CSF, from patients undergoing psychosurgery, of amino acids with actions at the N-methyl-D-aspartate receptor. The only significant difference ($P<0.05$, Student's t-test) between patients receiving (left-hand columns) and not receiving antidepressants (right-hand columns) was for serine. For the 12 neurological patients, the nine Alzheimer patients, and the other seven demented patients the respective mean (s.e.) aspartate values (nmol/ml) were 1.24 (0.07), 1.17 (0.12) and 1.43 (0.11); for glutamate 1.38 (0.13), 1.46 (0.16), and 1.66 (0.39); and for serine 49.5 (4.7), 44.5 (3.4), and 46.7 (4.6).

At the time of the operation 14 subjects had been receiving antidepressants for at least two weeks. On a range of demographic and clinical features, as well as storage time of samples (data not shown), patients did not differ, with few exceptions, from the other 17 patients. These had had no antidepressants for six months, except for four who had been free for at least two weeks (Table 1).

Figure 1 shows that aspartate and glutamate values were distributed over relatively wide ranges, particularly in subjects not taking antidepressants. When these subjects were compared with those on antidepressants, no significant differences were found in aspartate and glutamate concentrations; this was also the case for asparagine (7.1 (0.6) nmol/ml for those taking antidepressants v.

Table 1
Details of patients included in the main study

	Patients receiving antidepressants in last two weeks	Patients free from antidepressants for six months[1]
Mean (s.e.) age: years	51.7 (3.5)	48.3 (3.3)
No. of men	5	9
No. of women	9	6
Mean (s.e.) disease duration: years	16.9 (2.6)	15.8 (2.4)
Diagnosis: %		
unipolar	50	41
unipolar, psychotic	14	12
bipolar	21	29
other	14	18
Other recent treatment[2]: %		
Drug free	0	18
Antipsychotics	14	35
Tranquillisers	57	59
Lithium	14	12
ECT	43	71
Mean (s.e.) score on the Hamilton Rating Scale for depression	21.9 (1.9)	19.4 (1.7)
Outcome: %		
well	8	13
minor symptoms	8	33
some improvement	54	33
no change	31	20

1. Except for 4 who had been antidepressant-free for at least 2 weeks.
2. During the 6 months, or for ECT 12 months, preceding collection of sample.

6.4 (0.4) nmol/ml for those not) and glutamine (488 (5) nmol/ml v. 484 (4) nmol/ml, respectively). Whereas the serine value was significantly higher in those taking antidepressants (Fig. 1; the mean serine value of the four who had been free of antidepressants for less than six months but at least two weeks was 36.2 (1.3) nmol/ml, which was even lower than that of all those not taking antidepressants). The groups were not exactly equivalent: a higher proportion of subjects free of antidepressants were men and more had recently taken antipsychotic drugs. These factors were without effect on serine values and those of the other investigated compounds, with the exception that glutamine levels were lower in women (data not shown) and patients treated with antipsychotic drugs (see above). The subjects free of antidepressants had also had more ECT. When analysis was confined to the 13 subjects who had had no recent ECT and drugs from two or fewer categories, none of the investigated compounds were affected by antidepressant treatment (taken by 8 of the 13) (data not shown). However, ECT is unlikely to explain the differences in serine values as the values for the 31 subjects receiving drugs from two or fewer categories were 38.9 (0.9) nmol/ml for 18 patients treated with ECT and 40.0 (1.0) nmol/ml for 13 free of this therapy (not significant, Student's *t*-test).

The difference in serine concentration alone which was associated with antidepressant treatment was a consistent finding, based on other comparisons. When analysis was confined to subjects who had recently received ECT and drugs from two or fewer categories, the serine contents were 41.5 (1.9) nmol/ml and 37.5 (0.7) nmol/ml for six patients receiving antidepressants and the 12 patients not receiving them, respectively ($P<0.05$, Student's *t*-test). Serine values for all 52 subjects were 40.7 (0.7) nmol/ml and 38.4 (0.6) nmol/ml for the 32 receiving antidepressants and the 20 other patients, respectively ($P<0.02$, Student's *t*-test). Content of glutamate (and aspartate) were unaffected by antidepressant treatment; values were 1.5 (0.1) nmol/ml (aspartate 1.1 (0.1) nmol/ml) and 1.5 (0.2) nmol/ml (aspartate 1.5 (0.2) nmol/ml) for those taking and not taking such medication, respectively. Demographic and clinical features of the two larger groups were similar to those of the groups in Table 1 (except for multiple drug treatment).

Patients not taking antidepressants appeared to have a better outcome one year after the operation when subjects receiving drugs from two or fewer categories as well as all subjects were considered. Of the 40 subjects examined at follow-up, 17 had been free of antidepressants at the operation and 9 (53%) of these were well or had only minor symptoms (categories I, II and III; Poynton *et al*, 1988) whereas 22% of those taking antidepressants were in these categories ($\chi^2=4.183$, $P<0.05$).

Discussion

The study of the role of excitotoxic mechanisms in human brain diseases is difficult (Procter *et al*, 1988). This study of ventricular fluid from anaesthetised patients with affective disorders and no structural evidence of altered CSF dynamics offers an approach with the advantage that it is free from the influence of the spinal cord, unlike the study of lumbar fluid. This is especially important when serine is studied, as it is one of the few amino acids found at a higher concentration in ventricular than lumbar fluid, suggesting it originates centrally. This may also explain why lumbar concentrations are high in the newborn if no correction is made for height.

To our knowledge there are only three other studies, all of samples from patients with various neurological disorders, that report concentrations of the important neurotoxic amino acid glutamate in human ventricular fluid (Engelsen *et al*, 1985; Perschak *et al*, 1987; Crawford *et al*, 1988). The mean value in patients taking antidepressants (Fig. 1) is 48% lower than the lowest value previously recorded, but is in close agreement with values for the present groups of neurological patients. These previous series show aspartate values distributed over a wide range but the value of treated patients is, in general, lower. Moreover, the mean aspartate value of subjects taking antidepressants was also lower than for any of the present groups of neurological patients. Thus there is no evidence that these amino

acids implicated in excitotoxic brain damage are elevated in patients receiving antidepressants. All of the individual values for serine (Fig. 1) were, with one exception, lower than 46.9 nmol/ml, the overall mean value for neurological patients.

The main finding of this study is that patients who are unresponsive to psychotropic drugs, regardless of type, did not have higher aspartate or glutamate concentrations in ventricular fluid than those untreated. This is in contrast to the finding with antidepressants and serine, an amino acid which modulates the action of glutamate at one of its synaptic receptors. Before this can be confidently ascribed to the antidepressants, it is necessary not only to eliminate any effect of ECT but to also consider prescribing habits in the treatment of resistant affective disorders. The fact that patients treated with antidepressants at the time of operation may have a poorer outcome raises the possibility that low serine concentration in ventricular CSF identifies a subgroup with a good response to the operation. Further studies of lumbar CSF would indicate whether this could be developed into a clinically useful procedure.

An alternative explanation is that the antidepressants themselves influence serine concentration by an as yet unidentified mechanism. However, the fact that subjects treated with antidepressants remained depressed indicates that this is independent of the patient's mood state. The neurobiological consequences of the altered serine concentration are also unknown (Johnson & Ascher, 1987; Procter *et al*, 1989; Marvizon & Skolnick, 1990; Procter *et al*, 1991) but in view of the long history of antidepressant use without serious sequelae becoming obvious it is unlikely to be deleterious (Dolan *et al*, 1986). Indeed, although ventricular enlargement in psychiatric illnesses has been attributed by some to treatments and possibly excitotoxic damage (Wood *et al*, 1990), these findings have also been described in patients dying before the introduction of antidepressant medications (Corsellis, 1962).

Acknowledgements

We gratefully acknowledge the cooperation of the large number of people involved in collection and classification of the samples, especially Dr D. M. A. Mann and Prof. D. Neary, and Mrs J. Damji for help in preparing the manuscript. We are grateful for financial support from Astra Alab, Haley-Stewart Trust, and the Science and Engineering Research Council. PHFB received an award from the Conselho Nacional Para O Desenvolvimento Cientifico e Technologico (Brazil).

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