Tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human cerebrospinal fluid: interrelationships and the influence of age, sex, epilepsy and anticonvulsant drugs

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SUMMARY Tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid were measured in cerebrospinal fluid, taken during pneumoencephalography, from a large series of patients, the majority of whom were epileptics, most of them receiving anticonvulsants. CSF indoleacetic acid reflects CNS tryptamine metabolism in the same way that CSF 5-hydroxyindoleacetic acid reflects CNS 5-hydroxytryptamine metabolism. Our data suggest that (i) the brain tryptophan content is an important factor in the control of both 5-hydroxytryptamine and tryptamine synthesis (ii) brain 5-hydroxytryptamine metabolism exhibits a U-shaped relationship with age (iii) the mean brain tryptophan content and rate of 5-hydroxytryptamine metabolism are greater for women than men (iv) indoleamine metabolism is unaffected in untreated epileptics compared with non-epileptics, but anticonvulsant drugs decrease the rate of 5-hydroxytryptamine metabolism.

Measurement of amine related compounds in the cerebrospinal fluid (CSF) is the most commonly used method for studying biogenic amine metabolism in the human CNS. For the indoleamines, the concentration of tryptophan in the CSF is a reasonably good index of the CNS tryptophan content, while the concentration of 5-hydroxyindoleacetic acid (5HIAA) in the CSF reflects CNS 5-hydroxytryptamine (5HT) metabolism. Recently we have shown that indoleacetic acid (IAA) in CSF reflects CNS tryptamine metabolism. Tryptamine, a minor metabolite formed by the action of aromatic amino acid decarboxylase on tryptophan (fig 1), belongs to a class of compounds called the trace amines. The trace amines are chemically related to the catecholamines and 5HT but are present in brain in much smaller amounts. There is increasing interest in their functional significance and their possible role in the aetiology of neuropsychiatric disorders.

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Fig 1 The pathways of indoleamine metabolism.
We have now measured tryptophan, 5HIAA and IAA in the first (lumbar) and last (cisternal) CSF samples taken during diagnostic pneumoencephalography. This was done in order to determine what influence tryptophan availability, the age and sex of the patient, epilepsy, and anticonvulsant drugs might have on 5HT and tryptamine synthesis in the CNS of man.

Methods

CSF was collected from patients undergoing diagnostic pneumoencephalography at the Montreal Neurological Hospital. The procedure was done on the fasting patients, without premedication, between 9 am and 12 noon. The first 2 ml of CSF was used for routine diagnostic purposes and the next 2 to 6 ml were collected for analysis of indoles. This sample was derived from the lumbar sac, which in humans has a volume of about 15 ml, and will be referred to in this study as lumbar CSF. Oxygen was then injected until the lateral ventricles contained oxygen. Some CSF from this compartment was thus displaced down into the basal cisterns, and when most of the oxygen had been injected (average of 60 ml), a second sample of CSF (6 to 8 ml) was collected through the lumbar needle. This last sample consisted mainly of fluid that was originally in the basal cisterns and was displaced into the lumbar sac. It is referred to in this study as cisternal CSF. No more than 30 minutes separated the collection of lumbar and cisternal CSF. Both samples were allowed to drip directly from the needle into acid-washed tubes, and were stored at -70°C until the analyses were performed.

Indoles in CSF were measured by the method of Anderson and Purdy. This method involves direct injection of 10 to 50 μl of CSF into a high performance liquid chromatograph (HPLC), separation of the various compounds on a reverse-phase column (30 cm × 3-9 mm column of 10μ “µ-Bondapack C18” from Waters Associates Inc., Milford, MA, USA), and measurement of the fluorescence of the indole ring using a modified Amino Fluoromonitor (American Instrument Co, Silver Spring, MD, USA).

Patients

Measurements were made on samples from 83 patients whose age (mean ± SD) was 29-1 ± 13-5 years. For technical reasons not all the compounds were measured in all the samples. For some of the analyses of the data the patients were grouped into non-epileptics, untreated epileptics and treated epileptics. The most common diagnosis among the non-epileptics was pituitary chromophobe adenoma, but there were also patients with other tumours, with multiple sclerosis and other miscellaneous diagnoses. There have been reports that CSF 5HIAA is low in multiple sclerosis, but this is true only for chronic patients who are severely disabled. In the present study the patients with multiple sclerosis were at an early stage and pneumoencephalography was performed to rule out other diagnoses. For both lumbar and cisternal samples, the mean CSF 5HIAA concentration for the four patients with multiple sclerosis was less than half a standard deviation from the mean of the other non-epileptic patients. Thus, as CSF 5HIAA did not seem to be low in the patients with multiple sclerosis, they were included in the non-epileptic group. All the epileptics were suffering from complex partial seizures, although none of them had a seizure in the 24 hours preceding the pneumoencephalogram. They were admitted to hospital for investigation because of inadequate response to anticonvulsants and were therefore relatively homogeneous with respect to the severity of the disease. Some of the patients had their anticonvulsant medications withdrawn as part of their investigation in hospital, and had been off all anticonvulsants for at least one week before pneumoencephalography. They are referred to as untreated epileptics. The treated epileptics were receiving from one to three of the following drugs: carbamazepine, diphenylhydantoin, phenobarbital and primidone. For all patients at least one of the plasma drug levels was within the therapeutic range (4–10 mg/l, 5–20 mg/l, 10–40 mg/l and 5–11 mg/l respectively).

Results

Gradients of indoles in CSF

Values of tryptophan, 5HIAA and IAA were available for both lumbar and cisternal CSF in 69 patients. To determine whether there was a gradient in the concentration of these compounds between cisternal and lumbar compartments, a paired t-test was performed. No significant difference was found for tryptophan but the concentrations of 5HIAA (t = 4.19, p < 0.001) and IAA (t = 2.16, p < 0.05) were significantly higher in the cisternal compartment.

Table 1 presents measurements made on the CSF of three patients with block of CSF flow, in the IIIrd ventricle, the aqueduct of Sylvius, and the IVth ventricle respectively. 5HIAA measurements were made on the first two patients, and in both the cisternal level was lower than the lumbar level. This was also true for IAA in two of the three patients. In the patient who had higher IAA in the cisternal compartment,
CSF (number 1 in table 1) tryptophan was also higher in the cisternal sample.

Relationships between tryptophan, 5HIAA and IAA

Table 2 gives the correlation and partial correlation coefficients between tryptophan, 5HIAA and IAA in lumbar and cisternal CSF. In lumbar CSF, 5HIAA is significantly and positively correlated with both tryptophan and with IAA. When the partial correlation coefficients were determined these relationships remained, and the small non-significant correlation between tryptophan and IAA disappeared entirely. For cisternal CSF the situation was different. All three pairs were significantly positively correlated, and when the partial correlation coefficients were determined only the relationship between 5HIAA and IAA became non-significant. This suggests that the relationship between 5HIAA and IAA was dependent on variations in tryptophan in the cisternal, but not in lumbar, CSF. Correlation coefficients were also determined for the three pairs shown in table 2 for both lumbar and cisternal CSF in sub-groups (males, females, non-epileptics, untreated epileptics, and treated epileptics). For all these sub-groups the trends were the same as in table 2. However, the sample sizes and some of the correlation coefficients were smaller, and not all the correlations that were statistically significant in table 2 achieved significance at the 5% level in all the sub-groups.

Table 2  Interrelationships between tryptophan, 5HIAA, and IAA in lumbar and cisternal CSF

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lumbar (n = 67)</th>
<th>Cisternal (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation</td>
<td>Partial correlation</td>
</tr>
<tr>
<td>Tryptophan and 5HIAA</td>
<td>0.46*</td>
<td>0.42*</td>
</tr>
<tr>
<td>Tryptophan and IAA</td>
<td>0.22*</td>
<td>0.00</td>
</tr>
<tr>
<td>5HIAA and IAA</td>
<td>0.48*</td>
<td>0.44*</td>
</tr>
</tbody>
</table>

The values are correlation coefficients between the variables shown. The partial correlation coefficient between two variables were calculated in order to determine the relationship between the two variables, eliminating the effect of the third variable. Significantly related at †p < 0.05; *p < 0.01.

Age differences

The age range of the patients was from four to 70 years. To determine whether there was any relationship between CSF tryptophan, 5HIAA or IAA and age we determined correlation coefficients between these three indoles, using both their lumbar and cisternal CSF concentrations and age, in both the whole group and in males or females only. None of these correlations were significant. However, inspection of a plot of cisternal CSF 5HIAA and age (fig 2) suggested that there is a higher order relationship between these two variables. Statistical analysis, performed as described in the caption to fig 2, revealed a significant quadratic relationship. The U-shape of the curve is not very pronounced, and the difference between maximum and minimum 5HIAA values on the line over the age range 15 to 50 years, which includes the majority of the measurements, is only 4.4 μg/l. Thus the age-dependent variation is very small compared with the total variation in 5HIAA values. No curvilinear relationship was found between 5HIAA and age in lumbar

![Fig 2](http://jnnp.bmj.com/)

**Fig 2**  The relationship between age of patients (yr) and cisternal CSF 5HIAA (ng/ml). Curvilinear regression was used to fit a second degree polynomial to the data. The significance of the quadratic term was assessed after the contribution of the linear term alone had been determined. There was a statistically significant (p < 0.05) quadratic relationship between cisternal CSF 5HIAA and age. The line of best fit, which is shown in the figure, is described by the equation y = 41.0 - 0.9347x + 0.01426x².
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CSF, or between tryptophan or IAA and age in either CSF compartment.

Sex differences
Table 3 shows the concentrations of indoles in the CSF of males and females. All the mean values are higher for females than males but this is only statistically significant for tryptophan and 5HIAA in cisternal CSF and for 5HIAA and IAA in the lumbar compartment. Thus, as with the interrelationships between the indoles, results are different in the lumbar and cisternal CSF compartments. The differences between males and females, that are seen for the whole group, were also found when the sample was divided into non-epileptics, untreated epileptics and treated epileptics. However, for these sub-populations the groups were smaller and none of the differences between males and females were significant at the 5% level.

Epilepsy and anticonvulsant drugs
Table 4 shows the effect of epilepsy and anticonvulsant drugs on indoles in CSF. There are no significant differences between non-epileptics and untreated epileptics in either lumbar or cisternal CSF. Anticonvulsant drugs seem to lower 5HIAA in lumbar CSF, as significantly less was found in samples from the treated epileptics than in samples from the untreated patients. This was not so for cisternal CSF, and there were no significant differences between the two groups in concentrations of tryptophan and IAA. The difference between lumbar CSF 5HIAA in untreated and treated epileptics was not due to a different sex distribution in the two groups, as there was a trend (p < 0.1) towards a similar difference when only the males or only the females were considered.

Discussion

Methodology
In this study we have looked at some of the factors that control the concentration of IAA in human CSF and reassessed how these factors control CSF 5HIAA. As will become apparent in the discussion below, our data on 5HIAA disagree with some results in the literature. One possible reason for these discrepancies concerns the methods used for CSF 5HIAA measurements. The original method developed by Ashcroft and Sharman12 employed fluorescence detection and was a notable achievement 20 years ago. However, comparison of this method with a mass spectrometric method indicates that it is subject to considerable errors.13 The method employed in this study involves a minimal amount of sample handling, and fluorescence detection is used only after the various indoles in CSF have been subjected to the powerful separating action of reverse-phase HPLC.9 Therefore, the accuracy of this method should be comparable to that of mass spectrometric methods, and preliminary data support this idea (GM Anderson, unpublished data). We feel that some of the discrepancies between results from this study and those in the literature may be explained by the greater scatter of points, resulting from the use of the less accurate simple fluorometric methods, in published studies. In the discussion below, the studies that are cited used variants of the simple fluorometric method unless otherwise specified.

The origin of indoles in CSF
The gradient we found for 5HIAA in CSF, with higher levels in the cisternal compartment, has been reported previously.24 The various factors that could account for such a gradient have been reviewed by Garelis et al14; they concluded that the most likely explanation is that the rate of synthesis of 5HT is higher in brain than in the spinal cord. In this study we confirmed, with a larger sample, our previous conclusion that IAA, like 5HIAA, exhibits a gradient in CSF24 indicating that the rate of tryptamine metabolism may be higher in brain
than in spinal cord. This conclusion is consistent with our data from patients with a block of CSF flow (table 1). In two patients the cisternal CSF 5HIAA is lower than the lumbar level, presumably because the obstruction in CSF flow from the ventricles to the basal cisterns reduces the 5HIAA in cisternal CSF and thus abolishes the gradient. A similar effect is seen for IAA in two of the three patients.

**Tryptophan availability and the control of indoleamine synthesis**

After oral tryptophan administration tryptophan and 5HIAA concentrations are increased in human lumbar CSF and show a significant and positive correlation. Thus, the significant positive correlations we found between these two indoles, in both lumbar and cisternal CSF (table 2), suggest that physiological variations in CNS tryptophan can influence CNS 5HT turnover in humans. Previous failures to find such a correlation in the lumbar compartment in the absence of tryptophan administration may have been due to smaller sample sizes and less accurate methods (see above). Curzon et al and Vapalahti et al found significant correlations in ventricular CSF, where 5HIAA levels are higher and hence the simple fluorometric assay would be more reliable. These data on ventricular CSF, together with our own data on cisternal and lumbar CSF (table 2) suggest that tryptophan availability does influence 5HT synthesis in the human CNS.

The relationship between tryptophan and IAA is more complex than that between tryptophan and 5HIAA. In cisternal CSF there is a significant positive correlation between tryptophan and IAA (table 2) and this fact, together with previous findings that CSF IAA varies sensitively with changes in CNS tryptophan, suggests that tryptophan availability influences not only 5HT but also tryptamine synthesis in human CNS.

**The influence of transport on amine metabolites in lumbar CSF**

The situation in lumbar CSF is not the same as in cisternal CSF. In the lumbar compartment the relationship between tryptophan and IAA disappears when the partial correlations are determined, while the significant relationship between 5HIAA and IAA, that we and Åsberg et al observed, remains (table 2). Thus, tryptophan availability does not appear to be the controlling factor for IAA in lumbar CSF (although it may still be involved in the control of spinal cord tryptamine synthesis). The most likely possibility is that IAA levels are strongly influenced by the CSF 5HIAA content, through competition for the transport system which removes these compounds from CSF. 5HIAA and other aromatic amino acids are removed from the CSF by probenecid-sensitive active transport systems which are present both in the choroid plexuses and in the cortical and spinal subarachnoid spaces. There is no evidence for such a transport system in the basal cisterns. Presumably therefore, the transport system will influence 5HIAA and IAA levels more in the lumbar CSF than in the cisternal CSF. This could explain why the correlation coefficient between tryptophan and 5HIAA is higher in the cisternal CSF than in the lumbar CSF. The competitive inhibition of one compound by another for the transport system out of CSF will be influenced by two factors, the concentrations of the compounds in CSF and their affinities for the transport system. Nothing is known about the relative affinities of 5HIAA and IAA for the transport system. However as IAA is present in smaller concentrations than 5HIAA in lumbar CSF, it may be that the 5HIAA will influence the CSF IAA level, through competitive inhibition of IAA transport out of CSF, but not vice versa. Our data suggest that transport has a more important influence on amine metabolite levels in lumbar CSF than in cisternal CSF. Thus, the basal lumbar CSF concentration of any acid metabolite of a biogenic amine that is present in small amounts (<5 ng/ml) probably does not reflect CNS metabolism of the parent compound unless the metabolite has a high enough affinity for the transport system so that it is not affected by competitive inhibition from other metabolites. Lumbar CSF IAA declines in depressed patients given nortriptyline, but this probably does not mean that nortriptyline influences CNS tryptamine metabolism; this change may be a consequence of the decline in CSF 5HIAA. As nortriptyline lowers CSF 5HIAA, possibly by decreasing 5HT turnover, there will be less inhibition of the transport of IAA out of the spinal subarachnoid space. The implication of this observation is that, to obtain the greatest information from measurements of trace amine metabolites in lumbar and ventricular CSF, the transport system should be blocked with probenecid. This probably does not apply to cisternal CSF, where our data suggest that transport is less important.

**Age differences**

Several studies have reported relationships between lumbar CSF 5HIAA and age. In three studies, 5HIAA increased significantly with age but in a fourth study a pronounced U-shaped relationship was found, with minimum values in the age range 40–50 years. The fact that we found no
relationship between age and lumbar CSF 5HIAA concentration in this study, with a large sample and using an accurate assay system, suggests that the increase with age reported previously may have been due to the fact that, with increasing age, there is an increase in CSF of compounds which interfere with the simple fluorometric assay. The quadratic relationship we found in the cisternal samples (fig 2), although not very pronounced, was statistically significant. Its presence in the cisternal rather than in the lumbar samples suggests that it reflects changes in the metabolism of 5HT with age, and not changes in the activity of the transport system which removes 5HIAA from the CSF. This view is supported by data from the study of Ashcroft et al.18 They found a quadratic relationship between lumbar CSF 5HIAA and age, very similar to that in fig 2, but only after the patients had received a load of tryptophan. If the quadratic relationship is due to a similar relationship between 5HT metabolism and age, then variation in one of two factors is presumably responsible, either the brain tryptophan content, or the activity of tryptophan hydroxylase. The absence of any relationship between CSF tryptophan and age in this study suggests that the enzyme activity might alter with age. The lack of any age-related change for IAA agrees with the results of Åsberg et al.23

**Sex differences**

Our data on sex-related differences in amine metabolites in CSF indicate that CNS 5HT metabolism is greater in women than in men (table 3). Gottfries et al27 found no difference between lumbar CSF 5HIAA in men and women. Åsberg et al.,24 using a mass spectrometric method, and Post and Goodwin,30 both found that the mean lumbar CSF 5HIAA of depressed women was 4–5 ng/ml higher than the mean for depressed men. However, with smaller groups than in this study these differences were not statistically significant. Post and Goodwin30 also measured CSF 5HIAA after probenecid administration. They found that the accumulation of 5HIAA was 50% greater in women than in men, a significant difference. In our study there was a difference in the mean values for men and women of about 6 ng/ml in both lumbar and cisternal samples (table 3). Wode-Helgødt and Sedvall31 found a significant negative correlation between height of the subjects and lumbar CSF 5HIAA concentration. They suggested that this relationship could be due to a greater surface area for acid metabolite transport in tall individuals. As men tend to be taller than women, this could explain part of the difference in CSF 5HIAA in lumbar CSF. However, we feel that a height difference is not the complete explanation. Firstly, although a height difference may show up in lumbar CSF it is unlikely to be seen in cisternal CSF. However, we did see a sex difference in the cisternal samples (table 3). Secondly, the height difference was attributed to a difference in the surface area for transport, whereas the sex difference was seen even when the transport system was blocked with probenecid.30 For these reasons we feel that the different CSF 5HIAA levels in men and women probably reflect primarily differences in CNS 5HT metabolism rather than differences in the transport system which removes 5HIAA from CSF. This is of interest because of the possible role of low brain 5HT in depression and the fact that the incidence of depression is greater, not smaller, in women than in men.

The fact that 5HIAA is higher in women can be explained partly by greater precursor availability, as the tryptophan level also is higher, and significantly so in the cisternal samples (table 3). However, it is unlikely that this is the only explanation, as the difference is proportionately greater for 5HIAA than for tryptophan, but it is not possible to say what other factors might be involved. Studies on experimental animals have revealed a variety of effects of gonadal hormones on the brain indoleamine system and, in the rat, brain 5HT is higher in females than in males.32 However, this difference was seen only on postnatal days 10 to 14.

**Epilepsy and anticonvulsant drugs**

Several groups have reported on CSF amine metabolite levels in epilepsy. Garelis and Sourkes8 found low levels of 5HIAA in CSF from epileptic patients (mainly on anticonvulsants) undergoing pneumoencephalography, compared with control values. Shaywitz et al33 found lower levels of 5HIAA in the lumbar CSF of epileptic children than in control children, after both groups were given probenecid. Most of the children were on anticonvulsants, but there was no relationship between plasma anticonvulsant levels and CSF 5HIAA. Chadwick et al30 measured both tryptophan and 5HIAA in lumbar CSF. They found that both compounds were normal in untreated epileptics. Anticonvulsants raised both compounds and particularly high values were seen in anticonvulsant-intoxicated patients. In a study on the metabolites of 5HT, dopamine and noradrenaline in lumbar CSF we found no significant difference between the concentrations of any of the three compounds in non-epileptics, untreated epileptics and treated epileptics.34 However the sample sizes were very much smaller than in the present study. Our present results (table 4) agree with those of Garelis and Sourkes8 and Shaywitz et al.,23 and suggest that the decline in CSF 5HIAA
is due to the anticonvulsant medication, not the disease. Possibly the difference between our results and those of Chadwick et al9 can be attributed to the methods used. In this respect, it should be noted that the mean value for CSF tryptophan in the control group of their study (778 ng/ml) is twice the value we found (380 ng/ml, table 4).

The mechanism by which anticonvulsants lower CSF 5HIAA is unknown, and it is surprising that this effect is seen only in lumbar CSF and not in the cisternal samples (table 4). One possible mechanism might involve folate metabolism. Anticonvulsant drugs often cause folate deficiency.36 Recently we have shown that lumbar CSF 5HIAA is low in patients who are folate deficient, due to dietary deficiency or malabsorption, and exhibit folate-responsive neuropsychiatric signs.36 Thus, the low CSF 5HIAA produced by anticonvulsants may be mediated by folate deficiency. This could be tested by measuring both 5HIAA and folate in the CSF of patients on anticonvulsants.

Conclusion

The data presented in this paper illustrate the advantages in using a precise method for quantitating several biochemically related compounds in CSF and applying it to samples from a large series of patients. It also illustrates the benefits of using samples taken during diagnostic pneumoencephalography when CSF derived from the basal cisterns, as well as the lumbar sac, can be obtained. In spite of the increasing use of computerised tomography, CSF is still available in many centres. Measurements on lumbar CSF sometimes provide more information on metabolism in the spinal cord than in the brain and our data indicate that the concentration of amine metabolites in the CSF can be influenced by transport more in the lumbar than in the cisternal compartment. Thus, the cisternal sample obtained in pneumoencephalography is particularly valuable. In this study we have used it to show that brain 5HT metabolism in humans can be influenced by age, sex and precursor availability, while brain tryptamine metabolism was affected only by availability of tryptophan.

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References

13. Sjöqvist B, Johansson B. A comparison between fluorometric and mass fragmentographic determinations homovanillic acid and 5-hydroxyindole-
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