Letters to the editor

‘Opioid peptides and dipeptidyl peptidase in autism’

SIR–We read with interest the paper by Hunter and colleagues 1 which proposes that previously published studies suggesting a role for opioid peptide in autism are ‘inaccurate’. They also suggest that their study ‘questions the validity of the opioid excess theory’.

It is encouraging that such theories are taken seriously and it is important that appropriate investigations are performed. It was particularly pleasing to see that detailed assessments of the study participants had been performed, as this is all too frequently ignored. It was also refreshing to see such a detailed description of the chemical analyses. Perhaps these details provide us with some clues as to why these results differ from those obtained by others. We would like to draw particular attention to the following points.

The table of results (Table II) on page 125 appears to be unrelated to the mass spectrometra it claims to record. The retention data differ markedly from those shown in the profiles – even the order of elution is different. It is difficult to see how conclusions can be drawn from such contradictory findings.

Most assays involving complex substrates, such as urine, consist of two stages. The preliminary preparation, or clean-up stage is designed to remove unwanted elements and to concentrate the material being identified or quantified in the following estimation stage. If the estimation stage is fairly non-specific (as with ultraviolet [UV] detection) then it is vital that the first clean-up stage is efficient. Where the final stage is very specific, as with the mass spectrometric (MS) methods, the requirements of the initial stage are less stringent. Hunter et al. used a clean-up process specific to an MS method2 that falls far short of that necessary for UV detection. For example, the crude extract is washed in a 0.15% acetonitrile solution, as against the standard 10% solution; and furthermore, they prepare their final extract in 100% acetonitrile solution, as against the standard 40% solution. The MS clean-up process leaves the urine largely unchanged and unsuitable for UV analysis. The result would be the uninterpretable chromatograms described and illustrated in this paper. Even in heavily spiked samples it would be impossible, as Hunter et al. admit, to identify individual peptides from such a mixture with UV analysis.

The concentration of bovine casomorphin used to spike the urine is not given, and its appearance in the MS spectrometra may reflect the technique’s greater sensitivity but might also be explained by the use of greater sample quantities: 560 microlitres for the (very sensitive) MS analysis compared to 40 microlitres for the (comparatively non-sensitive) UV method. To identify bovine casomorphins under these circumstances would be very difficult; to determine the very low levels of deltorphins or dermorphin which could be present would be very much more difficult still.

The authors failed to detect bovine casomorphin 1-7 in any of the 10 samples from children with autism (or from the sibling samples they used as controls). Like Dr Karl Reichelt (University of Oslo, personal communication), we detect this particular peptide in measurable quantities in only some 25–50% of our case series with an almost total bias towards those children whose autism has been present from a very early age. Of the 10 children, three were on diets devoid of casein (as well as gluten) and seven had undergone regression in their second year.

The introduction misattributes the advocacy of casein-free diets to Whiteley 3 and in support of its own failure to identify peptides quotes two earlier papers: Zhang’s paper 4 is a brief (200 word) abstract of a non-peer reviewed conference poster; the paper by Pavone and Fiumara 5 (actually published in 1997 and not 1996) discussed the relationship of autism with coeliac disease and reported on the absence of antibodies to gluten, but made no mention of peptides.

Given these significant methodological shortcomings, we question the validity of the findings and, therefore, the basis of the comments on the opioid excess theory of autism.

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References

‘O’Hare replies’

SIR–Table II and Figure 2 on page 125 of our paper 1 are both correct. However, we should have made it clearer in our labelling on page 125 and in the text on page 126 that Table II referred to data from a reference standard in water of the peptides, and that Figure 2 shows results from the spiked control urine sample.

The extraction method used by my colleagues and I was as described in the patent, 2 where they state that they are able to obtain UV chromatograms which would indicate the presence or absence of these opioid peptides. This method is also similar to the extraction method (‘preliminary clean-up’) which is detailed in Dr Shattock’s own paper. 3 However, using this method we have demonstrated complete recovery of peptides from spiked control urine samples; therefore, as Dr Shattock admits, the MS endpoint is considerably more sensitive and specific than the UV method. The absence of any peaks on the MS chromatogram would suggest the absence of these peptides at levels greater than 0.5mg on the column.

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(sample volume 20ml, concentration 25ng/ml).

To answer the third point, the amount of each peptide spike in 10ml of control urine was 1.25mg; two-fifths of the reconstituted sample were then analyzed. In order to allow the profile to stay within the scale of the MS, only 40ml of the extract was injected onto the UV system. To provide the best chance of observing peptides, the remainder of the extract (560µl) was then injected into and analyzed by the MS. In this way, it was hoped that both the profile and mass information could be obtained for each sample. We disagree with Dr Shattock, in that absence of any peaks for these peptides in the MS analysis, which is both more sensitive and more selective than the UV method, supports our evidence that these peptides were not present in the urine of children studied.

It is difficult to comment on the validity of the observation (attributed to personal communication cited in Shattock et al.3) that, the 25–30% of children shown by these research groups to have bovine casomorphin 1-7 in their urine, are different from our study group.

Whilst Whiteley4 does not study casein exclusion in his study, he comments in the abstract, introduction, and discussion on casein and gluten exclusion diets; thereby, suggesting that both food products are implicated. Pavone et al.5 should have been cited at the end of the sentence, whose ICP was normalized medically (combined acetazolamide and furosemide) and surgically (ventriculo-peritoneal shunting). Therefore, it is likely that the major impact of CPP will be during the acute stage of TBM before measures to reduce ICP are implemented.

We retrospectively reviewed the records of all children admitted to Tygerberg Hospital, South Africa with moderate to severe TBM (stages 2 and 3) between 1985 and 1995. Seventy of these patients were admitted to the present study on account of availability of data regarding ICP and CPP on admission. Parental informed consent was obtained in all patients as well as approval for the study from the Ethics Committee of the University of Stellenbosch.

Tuberculous meningitis was diagnosed whenever the history and cerebral spinal fluid (CSF) findings were typical of TBM and TB bacilli were isolated from the CSF or gastric aspirate. A diagnosis of probable TBM was made when children in the above-mentioned clinical context complied with two or more of the following: a strongly positive Mantoux test (>15mm), chest radiograph findings suggesting tuberculosis (i.e. miliary picture or hilar lymph adenopathy) often accompanied by segmental lesion and acute hydrocephalus with basal enhancement on computerized tomography.

The severity of the disease was classified according to the British Medical Research Council classification.10 All 70 patients had either stage 2 or 3 TBM on admission. The level of consciousness in stage 2 TBM ranged from sleepy and irritable to stuporous. These patients were all able to localize pain although some only unilaterally due to associated hemiparesis. Patients with stage 3 TBM, however, were deeply comatose and unable to localize pain. Many of these patients had associated brainstem signs and uni- or bilateral hemiparesis. The antituberculosis treatment consisted of daily rifampicin, isoniazid, ethionamide, and pyrazinamide (20, 20, and 40mg/kg/day respectively). All medication was given as a single dose before breakfast for six months. Drug compliance was carefully monitored because the patients were hospitalized for six months.

On admission the lumbar CSF pressure was monitored continuously for one hour by means of a Gaeltex transducer with a Luerlock fitting which was connected to a No 22 lumbar

References

‘Does cerebral perfusion pressure influence outcome in children with tuberculous meningitis?’

SIR—Despite the availability of adequate antituberculosis treatment for more than 30 years, the prognosis of tuberculous meningitis (TBM) remains poor. The outcome of TBM is known to be affected by age,1 stage of the disease on admission,1,2 and steroids.3

The various pathological mechanisms of brain damage in TBM have been reviewed by Dastur and Lalitha.4 Raised intracranial pressure (ICP) due to tuberculous hydrocephalus may potentially impede cerebral perfusion, while tuberculous periarteritis may cause cerebral ischaemia and infarction. Schoeman et al.2 found that the level of raised ICP on admission and outcome in patients with TBM did not correlate. Goiten et al.5 however, found that a cerebral perfusion pressure (CPP) below 30mmHg was always associated with death in both ischaemic and infectious conditions of the central nervous system. Some authors reported that raised ICP and low CPP indicate a poor prognosis in patients with near drowning6 while others found that some children who had a poor outcome did not have raised ICP.7 Minns and Barlow8 showed that a low CPP, but not mean or maximum ICP, related significantly to clinical outcome in non-accidental head injury.

All patients with TBM seen at our institution have active normalization of raised ICP, either by means of a ventriculo-peritoneal shunt (non-communicating hydrocephalus) or medical treatment (communicating hydrocephalus). Previous studies9 showed that ICP falls dramatically in the majority of TBM patients with communicating hydrocephalus within the first week of medical therapy. In addition, no significant difference could be demonstrated in the outcome of patients whose ICP was normalized medically (combined acetazolamide and furosemide) and surgically (ventriculo-peritoneal shunting). Therefore, it is likely that the major impact of CPP will be during the acute stage of TBM before measures to reduce ICP are implemented.

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puncture needle. The rest of the recording equipment consisted of a Gaeltec pre-amplifier connected to a pen recorder. The mean ICP was calculated as the average of the baseline ICP, which was measured every 5 minutes during the hour of continuous ICP measurement. During the pressure recording the blood pressure was measured every two minutes using a Dynamap apparatus. The mean of each blood pressure reading was calculated, and the mean of all these measurements was used as mean blood pressure. Mean CPP was then calculated by subtracting the mean ICP from the mean blood pressure. Patients with incomplete data and non-communicating hydrocephalus were excluded from the study. Communicating hydrocephalus was diagnosed by air encephalogram: 10ml of air was injected into the lumbar CSF space at the end of the first pressure recording. Demonstration of air in the ventricles on a skull radiograph, following the above procedure, was regarded as indicative of communicating hydrocephalus. Lumbar and ventricular CSF pressure is generally accepted to be equal in communicating hydrocephalus.11

Patients included in this study were drawn from our previous studies, which compared different methods of normalizing raised ICP secondary to communicating hydrocephalus in TBM. Most patients received oral acetazolamide (50–100 mg/kg/day) and furosemide (1mg/kg/day) which we have previously shown12 to normalize ICP in 78% of cases of TBM within the first month of treatment. The effect of this treatment on ICP was assessed by weekly continuous 1 hour lumbar CSF pressure recordings during the first month of treatment. Whenever medical treatment failed to normalize ICP within the first month of treatment, a ventriculo-peritoneal shunt was inserted. The clinical outcome of these patients did not differ significantly from that of patients with non-communicating hydrocephalus who had a shunt put in straight away.12

Clinical outcome was measured in all surviving children after 6 months of antituberculosis therapy. Intelligence was measured by a clinical psychologist using the Griffith Scales of Infant Development. Hearing was tested by means of free field audiometry or brainstem audiometry when indicated. Vision was tested by an ophthalmologist, and motor function by a neurologist. The following scale which assessed outcome on account of motor deficit, vision and hearing loss, and IQ was used: Normal − IQ>75, vision and hearing normal, no motor deficit; Mildly disabled − IQ 55−75, and/or decreased vision, and/or decreased hearing, and/or hemiparesis; Severely disabled − IQ<55 and/or blindness, and/or deafness, and/or quadriplegia death.

The Kruskal-Wallis Test was used to assess the relationship between CPP and outcome. A p value of <0.05 was indicative of statistical significance.

Mean age of the patients was 33 months (range 4–90 months, standard deviation 14 months). Thirty-six patients were male and 34 female. We found that 67 of the 70 patients had a CPP of less than 70mmHg on admission. The outcome score for the whole group showed no correlation between CPP values and clinical outcome.

Thirteen patients had a very low CPP (below 30mmHg) on admission. Two of these children died, three had mild and three severe neurological disability, and five were normal. All five children who were normal on follow-up, had stage 2 TBM and no clinical or computerized tomography evidence of cerebral infarction. On admission, the level of consciousness in these children ranged from sleepy with eye contact on arousal (n = 2), to stuporous with no fixating and following on arousal (n = 5). In contrast, focal neurological signs and computerized tomography features of cerebral infarction were present in all eight children with a poor outcome. Four of the these children had stage 5 TBM and deep coma on admission, while four others with stage 2 TBM were sleepy but arousable on admission.

A CPP of 70mmHg or more is regarded by neurosurgical critical care experts as indicative of adequate cerebral perfusion.13 A study from Guy’s Hospital, London, UK, showed that this CPP value also applies to non-traumatic coma in children.14 The reason for this critical CPP not applying to TBM probably relates to pathological differences between TBM and the above-mentioned conditions. All children with TBM included in the present study had raised ICP due to communicating hydrocephalus, a condition uncommonly associated with cerebral herniation. In addition, a low CPP in TBM is almost always due to raised ICP and not hypotension, as is often the case in traumatic and other causes of non-traumatic coma. The relative contribution of blood pressure and ICP to CPP may, therefore, be important when determining a critical CPP value in children.

In conclusion, we found that neurological outcome in TBM related to focal neurological signs and evidence from computerized tomography of cerebral infarction, rather than to CPP on admission. We believe that until the effect of a low CPP on existing ischaemic brain damage in TBM is known, the practice of fluid restriction for the syndrome of inappropriate antidiuretic hormone secretion in this condition should be reconsidered.

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References


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