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S24

Can the pathophysiology of autism be explained by the nature of the discovered urine peptides and dietary antigens?

K.L. Reichelt
Oslo University, Blindern, Oslo and lab 1, Sandvika, Norway

Purpose A: 1. To develop the urine analysis for exorphins for routine use in blood and cerebrospinal fluid (CSF).
2. Disorders where patient related validation must be carried out: schizophrenia, depression (uni- and bipolar) and autism.
Method A: HPLC-MS/MS (fragmentation mass spectrometry) technology.
With both a specific HPLC retention time and MS/MS (fragmentation) this method is close to an absolute technique for peptide recognition.
B: ELISA against specific proteins (gliadin, gluten and casein and transglutaminase 6) (Table 1 og 2).
Background A: schizophrenia: increased opioid peptide levels have been found in Schizophrenia using HPLC, immune assay and behavioral tests. [1–6] as part of a general peptide increase in urine. Since peptides are signaling compounds inhibition of peptidases during transport and work up of samples is critical to prevent break down, which is as expected fast at room temperature.
Strongly supporting is view is the data on postpartum psychoses (a very symptom rich psychosis) where also amino acid sequence of human casomorphin found increased, has been done [7–8]. The opioids can explain most of the symptoms of the psychotic schizophrenic state [6]. It is of paramount importance then to measure these peptides in carefully diagnosed patients on and without medication, in urine, blood and spinal fluid.
As can be seen in Table 1, it is important to measure IgA and IgG antibodies against the precursor proteins for the exorphins, which are found increased by several groups, and also have direct effects on the nervous system [9].
B. In depression increase levels of peptides has been found [18,28,29] and also opioid levels measured as opium receptor binding peptides [28]. In schizoaffective psychosis MS/MS exact detection of exorphins have been published [6]. Also in this syndrome it is critical to be able to measure the exorphins in blood and CSF, especially since the peptidases involved in break down of exorphins are decreased in depressions [30,31]. Inflammatory interleukins are also increased in depressions both uni- and bipolar [32] indicative of inflammatory processes probably in the gut. Inflammatory interleukins increase the permeability of epithelial membranes [33].
C. Autism. Considerable work has been done using HPLC with UV detection and co-chromatography [12,34–40]. However, with HPLC–MS/MS we can ensure that we are measuring only the exorphins and not chromatographically similar peaks that hide inside the main peak [41–43]. We therefore need to validate the new method in autism for both urine, blood and CSF (CSF collected only when spinal tap has to be done in any case).
Inhibition of break down in urine, blood and cerebrospinal fluid (CSF)
After extensive testing we have been left with three inhibitors. Citric acid 0.2 M; acetic acid 0.2 M and aprotenine [44,45].
These body fluids will be provided by Prof Dr E. Severance and Prof Dr R. Yolken (Johns Hopkins Univ.) and Prof Dr. Cunningham (Uppsala Univ. Sweden). Lab 1 provides monovettes with citric acid as peptidase inhibitor for urine collection. Blood will be collected in EDTA – aprotenin vacuum test tubes (Vacutainer) as will be CSF. HPLC and MS/MS detection.
The amount of urine analyzed on the HPLC after work up = 250 nanomles creatinine. To pick out generally active peptides in any one disorder, five and five autistic children or schizophrenic derived and depressive derived urines are mixed, creatinine re-determined and rerun. Peaks that are common to all patients increase or remain the same, while individual peaks of material on the HPLC runs are diluted out.
The complete procedure is published in detail [48]. If we use reporter ions we do not have to match all the peaks as shown in attached figures. On Fig. 1, synthetic bovine β-casomorphine 1-4 (Y-P-F-P) is compared to biologically isolated compound from a batch of five autistic children. On Fig. 2, the faster routine analysis using reporter ions is shown for bovine β-casomorhne 1-4. Top trace is synthetic casomorphin 1-4 and bottom trace is biologically isolated compound. The complete analysis for a series of opioids is published [48].
Program is then in sequence:
– A: further validation of method for urine in the different disorders;
– B: validation of method for blood in the same disorders;
– C: validation of method for CSF (spinal fluid) in schizophrenics and depressive patients.
NB.
To avoid overlooking new compounds a complete HPLC run with UV 215 nm (peptide bonds); 280 nm (aromatic groups) and 325 nm (Indolyl-acryloid) shall be run for urines. If sufficient serum is available and spinal fluid these will also be run on HPLC in addition to MS/MS detection.
Antibody assays will be done at Johns Hopkins using ELISA, Transglutaminase 6 antibodies at Lab 1 also using ELISA assay.
Figures and references not available in the abstract.

Table 1 Antibodies of type IgA and IgG increased in relevant disorders.

Disorder	References
Autism spectrum	Reichelt et al. [10]; Lucarelli et al. [11]; Cade et al. [12]; Vojdani et al. [13]; Kawashti et al. [14]; Trajkowski et al. [15]; Lau et al. [16]; de Magistris et al. [17]
Depression	Sælid et al. [18]; Maes [19]
Bipolar	Severance et al. [20]
Schizophrenia	Dohan et al. [21]; Reichelt and Landmark [22]; Samaro et al. [23]; Dickerson et al. [24]; Severance et al. [25]; Jin et al. [26]; Niebuhret al. [27]

Reference no in parenthesis is found in the reference list. The antibodies are of the IgA and IgG type and not IgE often found in allergic pathology.

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S25

Gastroenterology issues in schizophrenia: Why the gut matters

E. Severance
Johns Hopkins University School of Medicine, Department of Pediatrics, Baltimore, MD, USA

Numerous risk factors for schizophrenia can be reconciled through a common enteric source. These risk factors include systemic