Review article: the concept of entero-colonic encephalopathy, autism and opioid receptor ligands

*Inflammatory Bowel Disease Study Group, †Centre for Gastroenterology and ‡Centre for Paediatric Gastroenterology, Royal Free and University College Medical School, London, UK; §Department of Pathology, Coombe Women’s Hospital and Trinity College, Dublin, Ireland

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SUMMARY
There is growing awareness that primary gastrointestinal pathology may play an important role in the inception and clinical expression of some childhood developmental disorders, including autism. In addition to frequent gastrointestinal symptoms, children with autism often manifest complex biochemical and immunological abnormalities. The gut–brain axis is central to certain encephalopathies of extra-cranial origin, hepatic encephalopathy being the best characterized.

Commonalities in the clinical characteristics of hepatic encephalopathy and a form of autism associated with developmental regression in an apparently previously normal child, accompanied by immune-mediated gastrointestinal pathology, have led to the proposal that there may be analogous mechanisms of toxic encephalopathy in patients with liver failure and some children with autism. Aberrations in opioid biochemistry are common to these two conditions, and there is evidence that opioid peptides may mediate certain aspects of the respective syndromes. The generation of plausible and testable hypotheses in this area may help to identify new treatment options in encephalopathies of extra-cranial origin.

Therapeutic targets for this autistic phenotype may include: modification of diet and entero-colonic microbial milieu in order to reduce toxin substrates, improve nutritional status and modify mucosal immunity; anti-inflammatory/immunomodulatory therapy; and specific treatment of dysmotility, focusing, for example, on the pharmacology of local opioid activity in the gut.

INTRODUCTION
Recognition that neuroactive compounds derived from the intestinal lumen can permeate the mucosa (healthy or diseased), cross the blood–brain barrier and cause psychiatric, cognitive and behavioural disturbances is not new; indeed, this axis is critical in oral medication of psychopathology. There is a growing awareness, particularly within the field of childhood developmental disorders, that, in a proportion of affected children, gut–brain interactions may contribute to abnormal neural development and the subsequent expression of aberrant behaviours. Difficulties in accepting the biological plausibility of such a model, particularly when attention has been focused upon primary pathogenetic mechanisms operating within the central nervous system, may reflect, in part, a perceived lack of an analogous gut–brain interaction in either human or experimental models of encephalopathy. Among gastroenterologists and hepatologists, however, the evidence for such a mechanism is readily apparent. It may help advance the argument by seeking analogy with circumstances in which there is clear evidence for an influence of the gut upon the normal brain.

Correspondence to: Dr A. J. Wakefield, Centre for Gastroenterology, Department of Medicine, Royal Free and University College Medical School (Royal Free Campus), Hampstead, London NW3 2QG, UK.
E-mail: wakers@aol.com

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HEPATIC ENCEPHALOPATHY

Hepatic encephalopathy—a variable impairment of cerebral functioning in patients with acute or chronic liver disease—is the result of multiple biochemical influences upon central neurotransmitter systems. In addition to the neurotoxic effects of ammonia, derangements in the γ-aminobutyric acid-ergic (GABA-ergic), serotonergic and dopaminergic systems are evident (reviewed by Albrecht & Jones and Butterworth). It may be more than just coincidence that changes in some of these neurotransmitter systems have also been described in autism.

Central to changes in the activity of these various systems in hepatic encephalopathy is a failure of the diseased liver to metabolize and eliminate toxic compounds derived from the gut. Hepatic encephalopathy may also be induced by porta-systemic shunting (re-routing the venous drainage of the gut to bypass the liver and enter the systemic circulation directly) for the amelioration of portal hypertension. Precipitants of hepatic encephalopathy in patients with liver disease include a high entero-luminal protein load, constipation and sepsis. The clinical management of hepatic encephalopathy includes restriction of dietary protein, prompt treatment of constipation and antibiotic therapy directed against luminal colonic bacteria. As such, hepatic encephalopathy represents a prototypic afferent gut–brain interaction that may provide insight into other encephalopathic states that have been linked to extra-cranial pathology. Are there circumstances in which primary gut pathology might also adversely affect the central nervous system?

ENTERO-COLONIC ENCEPHALOPATHY

Untreated coeliac disease—an aberrant immune response to dietary gliadin—is associated with intestinal mucosal inflammation, increased intestinal permeability, increased absorption and urinary excretion of neuroactive dietary peptides, and in some autistic and psychotic behaviours and neurological complications. Where coeliac disease is clinically silent due to a lack of gastrointestinal symptoms, a careful history often reveals that, in many such cases, there is low-intensity comorbid illness associated with decreased psychophysical well-being that includes, in children, behavioural disturbances with a tendency to depression, irritability and impaired school performance. Fabiani et al. described an increase in weight and height velocity, appetite, mood amelioration and an improvement in physical and school performance, in adolescents whose coeliac disease was apparently asymptomatic at diagnosis, once they began following a gluten-free diet. The precise mechanism(s) of behavioural and neurological sequelae in coeliac disease has not been established, although toxicity from the gut and autoimmunity are pathogenetic forerunners.

D-Lactic acidosis, a complication of acid-tolerant bacterial overgrowth in patients with short bowel syndrome and those undergoing intestinal bypass surgery for obesity, is associated with a range of psychiatric and neurological sequelae. Patients may present with altered mental state, aggression, stupor, ataxia and asterixis; these symptoms respond rapidly to oral antibiotic treatment. Encephalopathy is a recognized presenting feature of intestinal intussusception in infants, and, intriguingly, may be reversible with naloxone. Constipation alone may precipitate confusional states in the elderly.

THE GUT IN AUTISM: CLINICAL PRESENTATION AND SUBCLINICAL DISEASE

Recently, there has been growing interest in the role of the gut in developmental disorders, principally autism. Gastrointestinal symptoms are common in children with developmental disorders, a fact that was recognized by some of the earliest commentators on pathobiological mechanisms in autism; in 1986, Dohan was reported to have written: "K. Soddy (University College Hospital, London) wrote me that he noted that recurrent gastrointestinal upsets were a constant feature of autistic children and that, among other symptoms, the deteriorating autistic child often has acute diarrhoea." These observations feature prominently in parental accounts, but have been largely ignored in the autism literature. In a systematic analysis of an unselected population of 385 children on the autistic spectrum, clinically significant gastrointestinal symptoms occurred in 46%, compared with 10% of 97 developmentally normal paediatric controls (odds ratio, 7.4; confidence interval, 3.60–15.65; \( P < 0.0001 \)).

We have investigated gastrointestinal symptoms in over 150 autistic children and have reported our early experience. In this cohort of children, a period of initial normal development was followed by developmental regression and loss of acquired skills, consistent...
Intestinal permeability, as measured by urinary excretion of metabolically inert sugars after oral dosing, is a surrogate marker of mucosal integrity and is elevated in the presence of intestinal inflammation, as in Crohn’s disease and coeliac disease. D’Eufemia et al. reported that approximately half of a cohort of autistic children Without gastrointestinal symptoms had raised intestinal permeability. Increased intestinal permeability in children with autism has also been reported by Horvath et al.; we are currently attempting to reconcile the difficulties associated with obtaining a standard urine collection in affected children and, to date, have encountered shortcomings in the estimation of permeability on spot urine samples (A. J. Wakefield, unpublished observations, 2001). The precise role or frequency of increased paracellular permeability in children with autism is not known. In addition, it is not clear to what extent data derived from changes in the permeability of the epithelium to inert sugars are relevant to the absorption of neuropeptides, as discussed below. Nonetheless, D’Eufemia’s detection of aberrant intestinal permeability in asymptomatic autistic children indicates that reliance upon symptomatology will substantially underestimate the proportion of autistic individuals with possible gastrointestinal pathology. The situation may mirror the subclinical ‘iceberg’ effect that is evident for coeliac disease. Any underestimate in autism will be compounded by the combination of a raised pain threshold that may be present in affected children and by their restricted ability to communicate symptoms.

However, for many, the perception remains that intestinal symptoms are to be expected in children with developmental disorders, reflecting effect rather than primary gastrointestinal dysfunction. It is essential, as a first step, to ask whether these symptoms reflect underlying visceral pathology.

The identification of increased intestinal permeability in a patient is not a diagnostic end-point, but indicates the need for further detailed investigation. As a corollary to changes in intestinal permeability, there is compelling evidence that many children with autism and gut symptoms have demonstrable organic pathology of the gastrointestinal tract. We have recently described a characteristic pattern of intestinal pathology—ileocolonic lymphoid nodular hyperplasia and enterocolitis—in a cohort of autistic children. The endoscopic and histopathological characteristics of this condition have been reported in detail elsewhere. A comparison of the mucosal lesion in both the colon and small intestine with appropriate controls reveals a pathology that may be distinct from established forms of mucosal inflammation; briefly, the colonic lesion consists of a mucosal infiltrate of γδ T cells and CD8+ T cells, significantly in excess of that seen in either normal or disease control groups. Crypt cell proliferation is substantially enhanced and the epithelial basement membrane is thicker than in either normal or disease control groups. Neutrophil and eosinophil infiltration of the mucosa is evident. The absence on colonic epithelium of human leucocyte antigen, HLA-DR, in autistic children suggests a T-helper-2 (T H2)-dominated immune response. Studies of the corresponding small intestinal lesion also indicate a distinct cell-mediated immunopathology in which immune-mediated epithelial damage is predominant, serum immunoglobulin G colocalizes with complement (C1q) at the epithelial basolateral membrane and epithelial proliferation is grossly increased. This is not seen in either healthy children or those with cerebral palsy. The intestinal changes are consistent with an autoimmune pathology and, in view of the increasing evidence for gut epithelial dysfunction in autism, are indicative of a specific and possibly important lesion. Bellanti and colleagues have presented preliminary evidence of similar findings in some children with attention deficit hyperactivity disorder, suggesting that gastrointestinal pathology may be relevant to a broader spectrum of childhood developmental/behavioural disorders.

Horvath et al. have reported their findings in the upper gastrointestinal tract in 36 autistic children, whose symptoms included chronic diarrhea, gaseousness, abdominal discomfort and distension. They detected grade I–II reflux oesophagitis in 25 (69%), chronic
gastri
tis in 15 (42%) and chronic duodenitis with
associated Paneth cell hyperplasia in 24 (67%).
Digestive enzyme activity was low in 21 (58%) and
pancreatico-biliary fluid output in response to intravenous
secretin was raised in 27 (75%). Subsequent to
Horvath et al.’s early report, we have included upper
gastrointestinal endoscopy in our own routine assess-
ment of affected children and have obtained similar
findings.

In summary, within the autistic spectrum, there is a
substantial group of children presenting with what may
be a primary, immune-mediated, intestinal pathology.
The constellation of developmental disorder and gastro-
intestinal pathology (provisionally termed autistic
enterocolitis) combines the paradoxical elements of a
motility disorder (oesophageal reflux plus constipation
with spurious diarrhoea) and enterocolonic mucosal
inflammation (a feature more commonly associated with
frank diarrhoea). Understanding the neurochemical and
immune basis of any gut–brain interaction in autistic
enterocolitis may help to resolve this paradox and
develop rational therapeutic approaches.

During the course of the clinical assessment and
management of these children, we have been impressed
by the symptomatic improvement in their behaviour
and general well-being following bowel clearance prior
to colonoscopy, treatment of intestinal inflammation
with 5-aminosalicylate-based compounds or a poly-
meric diet,35 relief of chronic constipation and, in
particular, the elimination of certain proteins (casein
and/or gluten) from the diet. Bolte has proposed a role
for intestinal clostridial dysbiosis in autism, specifically
via neurotoxic encephalopathy.30 In seeking to test this
hypothesis, Sandler et al. noted objective cognitive
improvement in autistic children in an open-label study
of oral vancomycin,37 an antibiotic that exhibits
minimal systemic absorption. Children regressed follow-
ing the cessation of therapy, suggesting that any colonic
dysbiosis and associated toxic sequelae were likely to be
secondary to the underlying intestinal pathology rather
than the primary problem. It seems likely that the
evidently dysfunctional mucosal immune system in
these children creates an environment that favours
anaerobic dysbiosis which is ameliorated only tempor-
arily by vancomycin. The findings of Bolte and
colleagues, although observed in an open-label study,
were largely unexpected. They are, in addition,
reminiscent of certain aspects of the clinical course
of hepatic encephalopathy, where oral, non-absorbable
antibiotic treatment is a first-line therapy. Is it possible
that an analogous interaction might be operating in a
subset of autistic children? If so, clues to any biochemi-
cal basis for such interactions in autism might be
identifiable in syndromes such as hepatic encephalop-
athy.

AUTISM AND OPIOID EXCESS

It has been proposed that some forms of autism may
arise from the toxic effects of intestinal products on the
developing brain. Kalat,38 Dohan,39 Panksepp,40
Reichelt et al.,41 Shattock et al.5 and Sun et al.42,43
have proposed that autism may be caused by inappro-
priate central activity of dietary-derived opioid peptides
(exorphins) from the gut. These include gliadmorphine
and b-caseomorphine from the substrates wheat gliadin
and bovine casein, respectively. Under normal circum-
stances, these abundant dietary opioids are digested by
brush border peptidases, such as dipeptidyl peptidase IV.

Intestinal inflammation is associated with increased
epithelial permeability that might facilitate the absorp-
tion of dietary peptides, as in coeliac disease,9 leading to
systemic opioid excess. Intestinal inflammation is also
associated with impaired digestive enzyme activity, as
shown for hydrolase activity in autistic children by
Horvath et al.26 and dipeptidyl peptidase IV in coeliac
disease.44 Diminished peptide hydrolysis and an
increased luminal opioid load may contribute to any
systemic excess. Once exorphins from the intestinal
lumen become systemic, the potential exists for them to
exert direct effects within the central nervous system,
and also to act indirectly by influencing the activity or
breakdown of endogenous opioids (endorphins) by
inhibition of tissue endopeptidases.45–49

In our cohort of autistic children, opioid-mediated
intestinal dysmotility would provide a rational explan-
tion for the clinical paradox of an enterocolitis
associated with chronic constipation and reflux oesoph-
agitis. Opioids exert potent effects upon motor and
secretory activity in the gastrointestinal tract, increas-
ing the gastric emptying time and inducing changes in
colonic myo-electrical activity, with concomitant
spasm, pain and constipation.50

In the central nervous system, exposure to opioid
excess during a critical phase of early cerebral develop-
ment may not only adversely influence this develop-
ment, but also increase the long-term susceptibility to
systemic opioids, whether exogenous or endogenous in
OPIOIDS AND DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

There is clear evidence from model systems that, in the developing brain, endogenous opioids and opioid receptors play a central role in neuro-ontogeny. Moreover, studies of prenatal drug abuse confirm that exogenous opioids can permanently perturb the developing brain, leaving affected children with associated deficiencies, including problems with learning and interpersonal relations. In an extensive and elegant series of studies, Zagon and McLaughlin confirmed that opioids and their receptors interact during brain development, and that opioid systems play an integral role in cellular proliferation, migration and differentiation within the developing central nervous system. Factors that were crucial in defining the specific patterns of aberrant neural development in rodents included the timing of experimental opioid perturbation in relation to the stage of central nervous system development; the numbers of prenatally derived neural elements, such as cerebellar Purkinje cells, were not influenced by postnatal opioid antagonism, whereas the development and growth of dendritic spines in these same cells—largely postnatal events—were altered profoundly. This observation may have considerable importance for our understanding of the timing (and possibly identification) of aetiological events and the associated autistic phenotype, as indicated by Courchesne. Post-mortem studies of autistic brains, although limited by small numbers, have revealed that, consistent with Zagon and McLaughlin’s observations, abnormalities in cell number (particularly cerebellar Purkinje cells) and dendritic arborization (particularly within the hippocampus) were two prominent characteristics that distinguished those with autism from neurologically normal controls. It was concluded that brains from those with early infantile (Kanner) autism and from low functioning autists with ‘mental retardation’ exhibited changes that were consistent with an aetiological factor operating in utero. It would be interesting to compare the morphology in these historical cohorts with that of autistic patients who undergo regression following a period of normal development.

In the studies of Zagon and McLaughlin, the effects of opioid antagonists on central nervous system morphogenesis and function were dose dependent, and varied according to the kinetics of delivery of the opioid antagonist (continuous or intermittent). In turn, according to the timing, dose and mode of administration of opioid antagonist, specific patterns of aberrant neural development were reflected in characteristic and measurable changes in behavioural ontogeny.

Against this background, there are (in addition to the clear consequences of opioid abuse) clinical and experimental data suggesting that aberrant patterns of neural development and behaviour, including autism, may develop in association with exposure of the brain to opioid excess. Evidence for such an association includes: the detection of bovine \( \beta \)-caseomorphine-7 (exclusively of dietary origin) in the urine, blood and cerebrospinal fluid of patients with psychoses, including autism; the demonstration that, in an animal model, systemically administered \( \beta \)-caseomorphine-7 (or an active peptide derivative thereof) crosses the blood–brain barrier, binds to and induces early activation gene-like (C-Fos) reactivity in areas of the brain that are implicated in autism, and that this induction is attenuated or blocked in a region-specific manner by the pre-administration of the opioid antagonist naltrexone; the demonstration of profound dose-dependent behavioural changes in the same animal model following peripheral administration of \( \beta \)-caseomorphine-7 (also attenuated or prevented by naltrexone pre-treatment); and the reported therapeutic benefit of dietary exclusion of the opioid substrates in autistic children. Trials of the oral opioid antagonist, naltrexone, in autism have identified improvements, particularly in behavioural domains such as self-injury. Nonetheless, results have been mixed and ultimately inconclusive. When interpreting these studies, it is important to take into account the narrow range of therapeutic efficacy for naltrexone as an opioid antagonist, and its U-shaped dose–response curve that would predict a beneficial effect at low doses and either no effect or a paradoxical aggravation of symptoms at higher doses. Unfortunately, few studies appear to have considered the relevant pharmacodynamics of opioid antagonists in the design of such trials.

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More recently, using electro spray mass spectroscopy to examine the urine of autistic children, Friedman and colleagues have identified, in addition to caseomorphine, a number of interesting and potentially relevant molecules. These include deltorphine, a morphine modulating peptide, and dermorphine; the latter molecule—a potent hallucinogen—is intriguing as it contains a δ-alanine in the second position from the C-terminal. In such a configuration, dermorphine would be resistant to mammalian peptidases. In nature, it is found in the skin of non-captive South American poison dart frogs, where it may be a microbial product, providing possible clues to its origin in the urine of autistic children.

OPIOID PEPTIDES AND HEPATIC ENCEPHALOPATHY

Of specific interest within the context of the current review is the increasing awareness of a role for opioid peptides in mediating the central effects of hepatic encephalopathy. Plasma levels of the endorphins, methionine-enkephalin and leucine-enkephalin, are increased in patients with cirrhosis and methionine-enkephalin is elevated in patients with acute liver disease. Changes in central opioidergic activity have been reported in different experimental models of fulminant hepatic failure and porto-caval shunting. The amelioration of symptoms with opioid antagonists is of particular interest.

A prerequisite for the participation of opioids, particularly exorphins, in the pathogenesis of central nervous system changes associated with hepatic encephalopathy and autistic enterocolitis would be evidence of altered opioid activity in the cerebrospinal fluid. Changes in cerebrospinal fluid endorphin and/or exorphin activity might be anticipated in view of the potential for exorphins, such as bovine β-caseomorphine-7, both to enter the central nervous system and influence cerebrospinal fluid endorphin levels through inhibitory effects on tissue peptidase activity.

In hepatic encephalopathy, alterations in cerebrospinal fluid endorphin levels are well established. Yurdaydin et al. showed that plasma and cerebrospinal fluid levels of endogenous methionine-enkephalin were significantly greater in patients with hepatic encephalopathy compared with both healthy controls and patients with lumbar back pain. As far as we are aware, exorphins of dietary origin have not been sought in hepatic encephalopathy, although the beneficial effects of dietary protein restriction, in addition to reducing substrates for ammonia synthesis, provide an empirical rationale for looking.

The exorphin, bovine β-caseomorphine, which has been associated most consistently with autism, can cross the human blood–brain barrier; Nyberg et al. have identified dietary-derived bovine β-caseomorphine-8 in the cerebrospinal fluid of otherwise healthy lactating women and linked excessive levels to postpartum psychosis. Molecular specificity in support of this claim requires confirmation by mass spectroscopy. In addition, autism is associated with changes in cerebrospinal fluid endorphin levels; Gillberg et al. identified high levels of endorphin fraction II (as defined by receptor-based assays) in the cerebrospinal fluid of children with autism, which also correlated with a reduced sensitivity to pain, a common finding in these children. Gillberg et al.’s study did not characterize the relevant opioid(s) further, although fraction II peptides that have been identified in human cerebrospinal fluid are mainly C-terminal elongated enkephaly peptides, including methionine-enkephaline-lysine and methionine-enkephalin-arginine-6-phenylalanine, both of which derive from the common precursor proenkephalin.

Within the central nervous system, opioids coexist with and modulate the effects of a wide variety of other neurotransmitter systems, including GABA-ergic, serotoninergic, dopaminergic and noradrenergic systems, which have been implicated in the pathogenesis of hepatic encephalopathy and autism. Sun et al.’s study of the pharmacological effects of β-caseomorphine-7 in rat brain showed the induction of gene activation in areas known to be either sources or components of dopaminergic, serotoninergic and GABA-ergic pathways. The behavioural abnormalities noted in this rat model by the same authors following systemic opioid administration were consistent with overactivity of the dopaminergic system. Sun et al. concluded that uptake of β-caseomorphine-7 by areas of brain pertinent to autism supports the hypothesis that dietary gluten and casein could be a cause of this condition, and is consistent with clinical improvement of autistic children placed on dietary exclusion of these substrates.

FURTHER CONSEQUENCES OF IMPAIRED DETOXIFICATION MECHANISMS

Experience with hepatic encephalopathy has demonstrated that the effects of liver dysfunction upon
neurotransmitter biochemistry are complex, and that it is difficult to correlate derangements in any one system with the severity of the clinical syndrome. We should not expect it to be any less complex in autism if analogous mechanisms pertain. Once again, however, hepatic encephalopathy may provide some clues for the study of autism. For example, it is well documented that abnormalities of glutamate/glutamine metabolism are prominent in patients with hepatic encephalopathy and autism, including autistic children with intestinal mucosal immunopathology. Compared with the former pathology, however, little has been done to progress our understanding of the significance of these abnormalities in autism. Glutamate is an important central nervous system metabolite and a major excitatory neurotransmitter. Once released from the presynaptic nerve terminal, it causes an increase in sodium conduction at the postsynaptic membrane and neurone firing. Inactivation is mainly within the perisynaptic astrocyte where re-uptake is associated with the glial glutamate transporter-1 (GLT-1) system. Metabolism includes transamination with oxaloacetic acid, decarboxylation (to GABA) and the addition of an ammonia group to form glutamine—a reaction catalysed by glutamine synthetase. An additional source of central nervous system glutamate in hepatic encephalopathy is via ammonia, synthesized by colonic bacteria and the deamination of glutamine in the small intestine. Following passive diffusion across the mucosa, failure by the diseased liver to effect what should, under normal circumstances, be a high first-pass clearance, leads to excessive ammonia levels in the brain. The consequence of sustained elevation of central nervous system glutamate concentrations in hepatic encephalopathy complicating chronic liver disease is a shift towards inhibitory neurotransmission. Impaired uptake of glutamate into nerve endings and astrocytes leads to excessive extra-synaptic accumulation that, in turn, is associated with the down-regulation of the glutamate receptor and decreased glutaminergic tone. In both hepatic encephalopathy and autism, a number of similarities have been described, including a decrease in the GLT-1 level in astrocytes, an increase in systemic l-glutamic acid level and an associated decrease in glutamine, findings that are apparent in children with autistic enterocolitis. While the significance of these changes remains to be fully elucidated, decreasing the substrate for glutamate synthesis, by reducing the availability, for example, of ammonia from the gut, is of therapeutic benefit in hepatic encephalopathy. While, in children with autistic enterocolitis, it is the integrity of the intestinal mucosa rather than the liver that appears to be at fault, understanding any associated derangements in amino acid biochemistry might allow a similar rational therapeutic approach. Our understanding of autism might also benefit from further insights provided by hepatic encephalopathy, including the observations of up-regulated benzodiazepine receptors and associated increases in the inhibitory neurotransmitter GABA. Conversely, for understanding hepatic encephalopathy, the analysis of certain biochemical derangements that have been reported in autism, such as defective sulpho-conjugation, may be a productive line of investigation. Sulphotransferase-mediated sulphation of phenolic amines is an important detoxification system; Waring and colleagues have reported abnormal sulphate metabolism with associated sulphate deficiency in autistic children. This may impair their ability to metabolize phenolic amines such as serotonin—raised levels of which have been described in autistic children—possibly contributing to neurological and behavioural symptoms. Impaired sulphation capacity may also contribute to abnormalities of the mucosal intercellular matrix and luminal mucins, which may be an important factor in epithelial dysfunction, as in inflammatory bowel disease.

**WINDOWS OF VULNERABILITY AND CLINICAL COURSE OF DISEASE**

From a clinical perspective, it may be argued that hepatic encephalopathy, unless prolonged, is fully reversible, whereas autism is not. However, the extent to which hepatic encephalopathy is reversed depends entirely on the institution of appropriate therapy that may include liver transplantation. Predicting a similar therapeutic response in the relevant subset of children with autism would require a much better understanding of the associated biochemical derangements. For example, some children with autism may exhibit behavioural/developmental improvements when placed on a diet that excludes certain opioid substrates, but tend to plateau at a level that is still demonstrably autistic. As illustrated above, it is likely that, in the relevant subset of children with autism, influential exogenous neurotoxins would probably consist of more than just casein- and gliadin-derived opioids and...
therefore the ameliorative effect of excluding these substrates would be only partial. Alternatively, although not exclusively, as opioid peptides are intimately involved in the regulation of neural ontogeny from an early stage, an opioid excess at a critical phase of cerebral development may produce enduring cognitive deficits that are not fully corrected by subsequent dietary restriction. Logically, therefore, the window of vulnerability for sustaining permanent impairment or susceptibility might be a neurotoxic exposure, such as an opioid excess, during a time of critical neuronal development during the first years of life. In affected children, it might be anticipated that the clinical manifestations—the autistic phenotype—will reflect the state of central nervous system development at the time of neurotoxic exposure, as evidenced by Zagon and McLaughlin’s studies of the effect of opioid receptor blockade on neuro-ontogeny.52–58 The recent apparent upsurge in regressive autism (a phenotype specifically addressed for the first time in the new Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) revised criteria101) possibly reflects an exposure that is delayed beyond the period of normal cerebral development that is accompanied by the acquisition of certain skills and behaviours. A narrow window of susceptibility for the induction of aberrant neural development may go some way to explaining why older children with alternative mucosal pathology, e.g. Crohn’s disease and ulcerative colitis, the onset of which is unusual before 10 years of age, have not, until recently, been reported to be at excess risk of autistic spectrum disorders. It is interesting, however, that patients with these mucosal pathologies of later onset do manifest an excess of psychiatric and neurological problems104 which, in Crohn’s disease, may pre-date the onset of intestinal symptoms.105 Intriguingly, Ericsson et al. have recently identified a statistically significant excess risk for autistic spectrum disorder in children with perianal/fistulizing Crohn’s disease.106 Might a delayed entero-colonic encephalopathy be operating in such patients?

ALTERNATIVE GUT–BRAIN ASSOCIATIONS

The merits of proposed alternative mechanisms of cerebral injury should be considered. Autoimmunity is one such mechanism, in which any concurrent gut and brain pathologies might operate either independently, or as part of a systemic autoimmune disorder. Immunological abnormalities appear to be common findings in children with autism,107–110 with increasing evidence for a significant autoimmune contribution to disease pathogenesis,111–113 particularly in those with enterocolitis.31, 33 The mucosal lesion in the small and large intestine is consistent with an autoimmune pathology, and the presence, in some affected children, of antibodies to myelin basic protein,111 neurofilament protein112 and cerebrovascular endothelium113 suggests the possibility of cerebral damage due to an autoimmune response to structural components of the central nervous system. There are, however, several inconsistencies in this hypothesis that require explanation. In addition to being a likely grey matter—rather than white matter—disorder, autism is not progressive. This is in contrast to other chronic immunopathologies associated with the presence of anti-myelin basic protein antibodies, such as multiple sclerosis. Imaging and histopathological studies do not support an inflammatory central nervous system pathology in autism. As part of our initial investigations,24 we undertook cerebral magnetic resonance imaging, electroencephalography and biochemical analysis of cerebrospinal fluid: none of these investigations indicated cerebral inflammation that would be consistent with an autoimmune process, although a more subtle lesion remains a possibility. Alternatively, the finding of a variety of autoantibodies in affected children suggests that, due to underlying immune aberrations, they may overproduce such antibodies, but their pathogenetic significance, if any, has yet to be determined.

Finally, it would be naive to consider opioid-mediated effects and autoimmunity independently, without reference to the fact that there may be substantial cross talk between the two. Opioid peptides and their receptors have been widely implicated in the regulation of certain immune responses, including lymphocyte apoptosis.114 The in vivo activation of central and peripheral opioid receptors by, for example, β-endorphin or morphine leads to the suppression of cellular immune responses, including lymphocyte proliferation, natural killer cell activity, interleukin-2 and interferon-γ production.115 Conversely, the administration of opioid receptor antagonists in a murine model induces the stimulation of these same responses that is associated with a skewing of the T-helper cell response from T\textsubscript{H}2 to T\textsubscript{H}1.116 It is tempting to speculate that luminal exorphins, some of which may derive from intestinal microflora, may, under normal circumstances, play an ecological role by participating in the maintenance of
immunological tolerance within the mucosal immune system.

CONCLUSIONS

The biological plausibility that exogenous, gut-derived neurotoxins can enter the systemic circulation and, by operating during a critical window of vulnerability, damage the developing central nervous system and cause autism is widely accepted; sodium valproate and thalidomide taken orally by the mother during pregnancy can cause autism in the offspring. The route to the foetal brain involves the mother’s intestine. Could exogenous neurotoxic opioid peptides act as a natural teratogen. Exogenous opioids may act similarly through direct and indirect mechanisms. The diet is one source of abundant exogenous opioids (and other potential neurotoxins) that, in the presence of intestinal mucosal pathology, may be inadequately degraded. Through a combination of altered epithelial permeability and mass action, these peptides may enter the systemic circulation in excess, and are detectable in the urine of children with autism. Hepatic encephalopathy, where an important role for aberrant opioid chemistry is becoming increasingly apparent, provides a prototypic model of gut–brain interaction that may render analogous pathological and therapeutic insights for those children with autism and entero-colonic pathology.

The various concepts explored in this review are capable of generating an abundance of hypotheses, most of which are testable in clinical and laboratory settings. Initially, however, a comprehensive quantitative and qualitative characterization of opioid (endogenous and exogenous) profiles is merited, in the blood, urine and cerebrospinal fluid of phenotypically homogeneous groups of children with autism, both on and off exclusion diets, including matched controls.

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