

ORIGINAL ARTICLE

Brain Effects of Chronic IBD in Areas Abnormal in Autism and Treatment by Single Neuropeptides Secretin and Oxytocin

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Abstract

Recent research points to the connection between behavioral and gut disorders. Early adverse events are associated with inflammatory bowel disease (IBD). In animal models, maternal deprivation and social isolation predispose to gastric erosion and brain pathology. This study examined (1) brain effects of chronic gastrointestinal inflammation in a rat model of acquired IBD and (2) whether such changes are resolved by individual secretin (S) or oxytocin (OT) peptide treatment. Neurological manifestations of IBD were mapped by *c-fos* gene expression in male Sprague-Dawley rats ($n = 10$) with trinitrobenzene sulfonic acid (TNBS)-induced IBD vs controls ($n = 11$). IBD was characterized by moderate/severe infiltration of inflammatory cells 10 d after TNBS infusion. Age-matched pairs were processed for immunocytochemical detection of Fos, expressed when neurons are stimulated. S or OT (100 μ g/250 μ L saline) or equivolume saline was administered iv by Alzet pump for 20 d after disease onset. Degree of resolution of colitis-induced brain activation was assessed by *c-fos* expression, and mean numbers of Fos-immunoreactive nuclei for each group were compared using Independent Samples T-test. Chronic IBD activated periventricular gray, hypothalamic/visceral thalamic stress axes and cortical domains, and septal/preoptic/amygdala, brain areas abnormal in autism. Single peptide treatment with S or OT did not alter the effects of inflammation on the brain. Brain areas concomitantly activated by visceral inflammation are those often abnormal in autism, suggesting that IBD could be a model for testing treatments of autism. Other single and combined peptide treatments of IBD should be tested. The clinical implications for treating autism, IBD, and concomitant sickness behaviors with peptide therapy, with or without maternal nurturing as a natural equivalent, are presented.

Index Entries: Treatment; autism; IBD; peptides; oxytocin; secretin; inflammation; amygdala; hypothalamic stress axis conditioning.

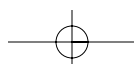
Introduction

Inflammatory Bowel Disease as a Model for Autism

Experimental models of visceral diseases, such as inflammatory bowel disease (IBD), might be useful

in testing novel therapeutic approaches for brain disorders associated with inflammation such as autism. This is the first use of IBD as an experimental model for autism. The relationship between autism and gastrointestinal (GI) disorders is poorly understood and remains in question. In the first author's clinical prac-

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tice, observations of socially isolated autistic children and maternally deprived orphans revealed shared symptoms of GI and neurologic manifestations, both of which were reduced by intense restoration of components of maternal nurturing. The reduction in both types of symptoms led to a theory that predicts a common peptidergic mechanism that acts simultaneously on the gut and brain. This theory prompted a study of the brain effects of chronic GI inflammation and the treatment of such effects with peptides associated with maternal nurturing. A discussion of the literature from disparate fields important to the understanding of this study follows.

IBD and Autism

Several links have been established between autism and GI disorders. Abnormal function of the GI tract in autism has been reported (Horvath et al., 1998; Wakefield et al., 2000; Lightdale et al., 2001; White, 2003; Koves et al., 2004). Patients with IBD often demonstrate psychiatric symptoms (Ringel and Drossman, 2001, 2002). Both IBD and autism share a high incidence of familial autoimmune disorders, suggesting a genetic component (Comi et al., 1999; Sweeten et al., 2003; Wen and Fiocchi, 2004). The pathogenesis of both IBD and autism involves immune dysfunctions, including dysregulation of Th1/2-type cytokines (Kucharzik et al., 1997; Gupta et al., 1998; Dohi et al., 1999; Iijima et al., 1999; Licinio et al., 2002; DeFelice et al., 2003; Sweeten et al., 2004).

IBD and Maternal Deprivation

Links between IBD and maternal deprivation syndromes and social isolation stress have also been established. Maternal deprivation or social isolation predispose to gastric erosion and brain pathology in experimental models (Ackerman et al., 1978; Meaney et al., 1988; Uno et al., 1989). Early adverse events in human development are also associated with IBD (Ringel and Drossman, 2001).

IBD and autistic spectrum disorders are both exacerbated by social isolation stress. Socially isolated autistic children and maternally deprived orphans have been observed to share symptoms of GI and neurological manifestations (Welch et al., 2004d-f). Social isolation stress might dysregulate humoral and cellular immunity (Popovic et al., 1999). Socially insubordinate vervets and autistic children share social isolation, hippocampal deficits, and GI abnormalities (Bauman and Kemper, 1985; Uno et al., 1989; Saitoh et al., 1995, 2001; Aylward et al., 1999; Bauminger and Kasari, 2000; Lightdale et al., 2001;

White, 2003). Long-term alterations of visceral sensitivity and gut mucosal integrity are found in animal models of maternal deprivation, one of the most profound forms of social isolation (Barreau et al., 2004).

Peptides and IBD

There is evidence that peptides can ameliorate IBD. Two peptides, vasoactive intestinal peptide (VIP) and epidermal growth factor (EGF), have shown efficacy in treating inflammation of the gut in both animal models and clinical trials. Abad et al. (2003) have demonstrated that VIP reduces inflammation of the gut in a trinitrobenzene sulfonic acid (TNBS) model of colitis. In a clinical trial, 10 of 12 patients with mild to moderate ulcerative colitis remitted after a 14-d treatment with EGF daily enemas (Sinha et al., 2003). The brain effects were not examined in either study.

Peptides and Autism

Abnormally low levels of two neuropeptides, secretin (S [GI tract]) and oxytocin (OT [plasma]), have been identified in autistic children (Green et al., 2001; Gershon and D'Autreaux, personal communication). Exogenous OT was shown to ameliorate repetitive behaviors in autistic adults (Hollander et al., 2003). A single systemic dose of S, administered as a probe of abnormal GI function, transiently ameliorated symptoms of autism in three young boys (Horvath et al., 1998). Vasoactive intestinal peptide (VIP), a member of the S peptide family, is elevated in the serum of neonates later diagnosed with autism (Nelson et al., 2001). Vasoactive intestinal peptide (VIP) has also been shown to induce a Th1/2 shift in vitro (Delgado et al., 2000), a mechanism that could account for the Th1/2 shift in autism.

These abnormalities also could be related to a familial finding of elevated plasma levels of serotonin in autism (Cook, 1990; Leventhal et al., 1990; Yirmiya et al., 2001). Serotonin is a neurotransmitter that is increased in inflammation of the gut (El-Salhy et al., 1997; Linden et al., 2003). Secretin (S) cells of five mammalian species were immunoreactive for serotonin (Cetin, 1990), which stimulates OT expression in the hypothalamic paraventricular nucleus (PVH) and supraoptic nucleus (SON) (Vacher et al., 2002). Serotonin systems in the brain are dysregulated in autism, including abnormal serotonin metabolism and asymmetries of serotonin synthesis, in the frontal cortex, thalamus, and cerebellum (Chugani et al., 1997).

Autistic patients demonstrate symptoms and sites of pathology (Bauman and Kemper, 1985; Buchs-

baum et al., 2001) that respond to S and OT treatment, both in human subjects and animal models (Porges, 2001; Hollander et al., 2003; Welch et al., 2003a,b). Both S and OT activate the visceral thalamic and hypothalamic stress axes in studies that were designed to determine the basis for their behavioral regulatory actions (Cushing et al., 2003; Welch et al., 2003b,c). These peptides are synthesized by hypothalamic, as well as gut and vascular, cells in response to homeostatic challenges such as visceral stress (Bayliss and Starling, 1902; Jankowski et al., 2000; Welch et al., 2003b, 2004a).

Brain Regions Abnormal in Autism

In our prior study, brain regions activated by infusions of S into the lateral ventricle include the prefrontal and frontal cortex, piriform (PIR) cortex, subcortical outlets, thalamus, hypothalamus, and the amygdala (Welch et al., 2003a). These areas have been shown to be abnormal in autism (Bauman and Kemper, 1985; Chugani et al., 1997; Rumsey and Ernst, 2000; Buchsbaum et al., 2001; Howard et al., 2000; Sparks et al., 2002; Bauman and Kemper, 2003; Vargas et al., 2004). Whereas there is still much work to be done in elucidating the pathology of autism, there are brain abnormalities that seem to be common to many cases. The brains of subjects with autism appear to be overdeveloped in some areas and underdeveloped in others (Mash and Barkley, 2003). For example, autistic patients often show high levels of connectivity within localized regions, suggesting overgrowth during early stages of development, particularly in the cortex. However, levels of connectivity between different functional brain regions might be lower than normal, also indicating general disruptions during synaptogenesis (Belmonte et al., 2004).

One structure in the brain that has been implicated in the development of autism is the cerebellum, a key region for language processing, anticipatory and motor planning, mental imagery, and timed sequencing (Bauman and Kemper, 2003). In particular, studies of autistic patients have often noted a marked reduction of Purkinje cells within the cerebellum (Courchesne, 1991; Courchesne, 2004; Bauman and Kemper, 2003; Kern, 2003; Belmonte et al., 2004). More recently, a link has been made between a defect in the role of the neuropeptide S and its receptors. Koves et al. (2004) have found a high rate of S immunoreactivity in the Purkinje cells of the whole cerebellum and in some of the neurons of the central cerebellar nuclei.

Other structural abnormalities have been linked to specific symptomatic features of the disease. For example, autistic patients with language impairment have shown asymmetry reversal of the frontal language cortex, whereas autistic patients without language impairment do not demonstrate this abnormality to the same degree (De Fosse et al., 2004). Other studies involving specific brain lesions have revealed that some of the impairments in social cognition seen in patients with autism might result from dysfunction of the amygdala (Adolphs et al., 2002; Daenen et al., 2002), including larger amygdala volumes than control children (Schumann et al., 2004) and small tightly packed neurons in the entorhinal cortex and in the most replicated site of pathology in autism, the amygdala (Martin-Ruiz et al., 2004). Enhanced activation in the right pericentral and premotor cortex (Muller et al., 2004) and increases in white matter volume were found (Herbert et al., 2004). An active neuroinflammatory process in the cerebral cortex and white matter, and a patchy loss of neurons in the Purkinje cell layer in the cerebellum, indicate that innate neuroimmune reactions play a pathogenic role in some autistic patients (Vargas et al., 2004). In further support of the link between inflammation and autism, aberrant innate immune response against endotoxin, a product of the gut bacteria, is suggested by a study finding proinflammatory cytokine production in response to ingestion of common dietary proteins by autistic children (Jyonouchi et al., 2002).

Maternal Nurturing/Deprivation

Endogenous OT affects areas of social recognition and early environmental conditioning of stress adaptation patterns in animal models (Carter, 1998). OT is stimulated by the act of breastfeeding and in turn stimulates lactation (Hatton et al., 1992; Matthiesen et al., 2001; Pedersen and Boccia, 2002). OT influences the physiological state of the mother and her mothering patterns, which in turn influence her child's physiological state and behavior (Francis et al., 2002). The mechanisms by which this influence occurs are related to enhanced vagal control of cardiac reactivity found in lactating women. In contrast, increased sympathetic and decreased parasympathetic nervous system tone were demonstrated in postpartum nonlactating women (Altemus et al., 2001), suggesting that children reared without the synchronous attunement of nursing and holding would likely have increased sympa-

thetic and decreased parasympathetic tone—a likelihood supported by clinical observations of children adopted from orphanages. Many of these children have suffered hunger and starvation, conditions that cause hypersecretinemia (Thuesen et al., 1987). They might have experienced intermittent periods of hypersecretinemia during their early adverse rearing in orphanages too poor to provide for their nutritional needs. Hyperdrive of the S system might have led to long-term deleterious effects on the system itself and/or on its effector organs.

Use of IBD Model to Test Peptides

Taken together, these data support a possible peptidergic link between behavioral and gut disorders such as in IBD or autism. Consequently, experimental models of visceral diseases such as IBD might be useful in testing novel therapeutic approaches for brain disorders associated with inflammation such as autism. In the current study, chronic IBD is used for the first time as a treatment model for autism. The purpose of the study is to determine (1) whether IBD induced in rats is accompanied by regional brain changes in *c-fos* gene expression in areas abnormal in autism, and (2) if so, whether treatment with S or OT reduces inflammation-induced brain changes.

Materials and Methods

Inflammation of Gut and S or OT Treatment

Data were obtained from 21 adult male Sprague-Dawley rats, weighing 250–450 g, from Hilltop Lab Animals (Scottsdale, PA) and housed at the New York State Psychiatric Institute Housing Facility. This study was approved by the Institutional Animal Care and Utilization Committees of both Columbia University and the New York State Psychiatric Institute. All experimental procedures met federal and state regulations.

Surgical Procedures

Rats were anesthetized with ketamine and xylazine and administered enemas containing either phosphate-buffered saline (PBS) or a mixture of 0.5 mL of solution of TNBS dissolved in PBS (pH 7.2) in an equal volume of ethanol for a concentration of 2% TNBS in 50% ethanol (Dohi et al., 2000). On days 0, 3, and 7, the TNBS enema was administered by rectum via a glass microsyringe equipped with a gastric intubation needle.

Treatment

Prior to implantation of an Alzet pump (model no. 2002), used to deliver either control vehicle or peptides, animals were anesthetized with xylazine (7–10 mg/kg ip) and ketamine (60 mg/kg ip). A surgical incision was made in the peritoneum of an anesthetized animal, and the pump was installed. Wounds were sutured, anesthesia was discontinued, bacitracin was employed as a topical antiseptic jelly, and animals were returned to individual cages for care and observation during the postoperative period. Saline or peptide S or OT contents of the implanted Alzet pump were delivered by iv infusion into the femoral vein. Control animals received 250 μ L of saline and IBD control, and IBD experimental animals treated with either S or OT received 100 μ g of either peptide (S or OT) per 250 μ L of saline, in all cases for 20 d (end point) at a rate of 0.5 μ L/h.

Immunocytochemical Detection of Fos

Animals were euthanized at the treatment end point by rapid ip injection of xylazine and ketamine, followed by transcardial perfusion, sequentially with physiological heparinized saline and a 4% solution of paraformaldehyde in sodium phosphate buffer (pH 7.4). Whole brains were removed and cryoprotected. The forebrain was sectioned from the frontal pole to the mesodiencephalic junction. Identical procedures were followed in control and experimental animals. Tissue blocks were postfixed for 2–3 h in individual glass vials containing 4% paraformaldehyde in 0.1 M PBS (pH 7.4) and cryoprotected overnight at 4°C in a solution of 10% sucrose in 0.1 M PBS. Frozen sections were cut on a sliding microtome at 30 μ m in the transverse plane, and every fourth section was processed immunocytochemically for *c-fos* gene product. Fos, in conjunction with other proto-oncogene proteins, turns on late genes that lead to long-term functional and structural changes in the brain and gut (Morgan and Curran, 1991). Tissues from control and experimental animals were processed simultaneously in the same solutions to control for potential variability in immunocytochemistry. All incubations were carried out in separate test wells on a Thomas rotator table. Tissues were collected in 0.1 M PBS (pH 7.4) in spot test wells and washed in Tris-buffered saline (TBS) between each step. Nonspecific binding sites were blocked by preincubating for 30 min in 1% bovine serum albumin (BSA) in TBS. Tissue sections were incubated overnight at room temperature with primary rabbit antibodies to Fos (diluted 1:10,000)