

## Secretin: Hypothalamic Distribution and Hypothesized Neuroregulatory Role in Autism

M. G. Welch,<sup>1,2,4</sup> J. D. Keune,<sup>1,2</sup> T. B. Welch-Horan,<sup>1,2</sup> N. Anwar,<sup>1,2</sup>  
M. Anwar,<sup>1,2</sup> R. J. Ludwig,<sup>3</sup> and D. A. Ruggiero<sup>1,2,3</sup>

Received June 19, 2003; accepted July 18, 2003

### SUMMARY

1. This study aims (1) to determine whether secretin is synthesized centrally, specifically by the HPA axis and (2) to discuss, on the basis of the findings in this and previous studies, secretin's possible neuroregulatory role in autism.

2. An immunocytochemical technique with single-cell resolution was performed in 12 age/weight-matched male rats pretreated with stereotaxic microinjection of colchicine (0.6  $\mu\text{g}/\text{kg}$ ) or vehicle into the lateral ventricle. Following 2-day survival, rats were anesthetized and perfused for immunocytochemistry. Brain segments were blocked and alternate frozen 30- $\mu\text{m}$  sections incubated in rabbit antibodies against secretin, vasoactive intestinal peptide, glucagon, or pituitary-adenylate-cyclase-activating peptide. Adjacent sections were processed for Nissl stain. Preadsorption studies were performed with members of the secretin peptide family to demonstrate primary antibody specificity.

3. Specificity of secretin immunoreactivity (ir) was verified by clear-cut preadsorption control data and relatively high concentrations and distinct topographic localization of secretin ir to paraventricular/supraoptic and intercalated hypothalamic nuclei. Secretin levels were upregulated by colchicine, an exemplar of homeostatic stressors, as compared with low constitutive expression in untreated rats.

4. This study provides the first direct immunocytochemical demonstration of secretinergic immunoreactivity in the forebrain and offers evidence that the hypothalamus, like the gut, is capable of synthesizing secretin. Secretin's dual expression by gut and brain secretin cells, as well as its overlapping central distribution with other stress-adaptation neurohormones, especially oxytocin, indicates that it is stress-sensitive. A neuroregulatory relationship between the peripheral and central stress response systems is suggested, as is a dual role for secretin in conditioning both of those stress-adaptation systems. Colchicine-induced upregulation of secretin indicates that secretin may be synthesized on demand in response to stress, a possible mechanism of action that may underlie secretin's role in autism.

**KEY WORDS:** secretin; autism; HPA stress axis; secretinergic neurons; conditioned response; oxytocin; visceral; neuroregulatory.

<sup>1</sup>Laboratories of Childhood Regulatory Disorders and Behavioral Neuroanatomy, Columbia University College of Physicians and Surgeons, Division of Neuroscience, NYSPI, 1051 Riverside Drive, New York, New York.

<sup>2</sup>Department of Psychiatry, Columbia University College of Physicians and Surgeons, Division of Neuroscience, NYSPI, 1051 Riverside Drive, New York, New York.

<sup>3</sup>Department of Anatomy and Cell Biology, Columbia University College of Physicians and Surgeons, Division of Neuroscience, NYSPI, 1051 Riverside Drive, New York, New York.

<sup>4</sup>To whom correspondence should be addressed at Department of Psychiatry, Columbia University College of Physicians and Surgeons, Division of Neuroscience, NYSPI, 1051 Riverside Drive, New York, New York 10032; e-mail: mgw13@columbia.edu.

## INTRODUCTION

To date, there is no comprehensive treatment for the broad range of autistic symptomatology: seizures (Park, 2003); attentional/arousal dysregulation and ADHD (Booth *et al.*, 2003); obsessive–compulsive disorder (Hollander *et al.*, 2003a); stereotypies (Militerni *et al.*, 2002); social isolation (Iqbal, 2002); attachment disorders (Kobayashi *et al.*, 2001; Tinbergen and Tinbergen, 1983); face recognition deficits (Ogai *et al.*, 2003, Schultz *et al.*, 2003); gaze aversions (Richer and Coss 1976); gastrointestinal disorders (Gershon, personal communication, 2003; Horvath *et al.*, 1998; Horvath and Perman, 2002; Torrente *et al.*, 2002); and altered heart rate variability (Corona *et al.*, 1998; Graveling and Brooke, 1978). Current drugs directed at treating these symptoms have long-term side effects and efficacy not far above placebo rates (Posey and McDougle, 2000), resulting in motivation to seek new treatments. The development of novel therapeutic measures targeting root causes of autism can benefit from combining understanding obtained at the bedside with knowledge gained at the lab bench.

Clinical work, in collaboration with Nobel Laureate Niko Tinbergen (Tinbergen and Tinbergen, 1983, Welch, 1983a,b, 1989), has shown that autistic spectrum disorders are ameliorated by reinstating components of maternal nurturing, including the establishment of synchronous attunement between mother and child (Welch 1983a,b, 1987, 1988, 1989, Welch and Chaput, 1988). Mother–infant interaction appears to be a powerful stimulus to neuropeptide release (Matthiesen *et al.*, 2001). Brain–gut neuropeptides contribute to developmental neuroregulation of growth, differentiation and regeneration, and the control of hormone release (Houben and Deneff, 1994), as well as the resolution of visceral inflammation and brain activation in brain/gut dysregulation models (Welch *et al.*, 2003b). These studies led to the hypothesis that maternal nurturing, as well as interventions that effectively replicate it, involves ameliorative mechanisms that stimulate neuropeptide release.

Our clinical and experimental work tests the hypothesis that some childhood developmental abnormalities are spectrum disorders of brain/gut dysregulation that can be ameliorated by naturalistic and/or peptide therapy. Recent research has revealed pathology in the gastrointestinal tract of autistic children extending from the esophagus to the colon. This finding has led to investigations of impaired gut–immune system development, altered production of brain/gut peptides, increased intestinal mucosal permeability, and inflammation (Nelson *et al.*, 2001; Torrente *et al.*, 2002; Warren *et al.*, 1997; White, 2003). Other evidence has focused attention on the possibility of homeostatic imbalance, such as altered central and peripheral energy metabolism in autistic children (Chugani *et al.*, 1999), and dysregulation of peptide hormones that protect homeostasis (Gershon, personal communication, 2003; Hollander *et al.*, 2003b; Nelson *et al.*, 2001). It is our belief that effective clinical or pharmacokinetic intervention in autistic symptomatology will require a mechanism that acts simultaneously upon the mind–brain–body stress axis to reestablish homeostasis (Welch *et al.*, 2003b). Metabolic imbalances in autism have been defined via fMRI (Haznedar *et al.*, 2000). Naturalistic and/or peptide therapies (Hollander *et al.*, 2003b; Horvath *et al.*, 1998; Welch 1983a, b; Welch *et al.*, 2003b) will be effective to the extent that they address such imbalances.

Psychotherapeutic and pharmacologic measures (Diggle *et al.*, 2003; Langworthy-Lam *et al.*, 2002), including peptide neurohormone administration, have been attempted in autistic children, with limited outcomes (Coniglio *et al.*, 2001; Dunn-Geier *et al.*, 2000; Horvath *et al.*, 1998; Kern *et al.*, 2002; Koren, 2001; Lamson and Plaza, 2001; Owley *et al.*, 2001; Sandler *et al.*, 1999; Roberts *et al.*, 2001; Lightdale *et al.*, 2001). Research supports the importance of peptides in treating behavioral and developmental disorders in autistics: at the bedside, through systemic peptide administration (Hollander *et al.*, 2003b; Horvath *et al.*, 1998), in clinical studies (Matthiesen *et al.*, 2001), and in experimental animals, through reinstating components of maternal nurturing, such as feeding, handling, and licking (Bredy *et al.*, 2003; Francis *et al.*, 2002). Experimental models show that feeding and handling ameliorate brain pathology resulting from the social-isolation stress of maternal deprivation (Anisman *et al.*, 1998; Meaney *et al.*, 1988, 1991). One peptide in particular, secretin, is associated with feeding and handling, a form of controlled restraint (Lauterbach *et al.*, 1980, Mineo *et al.*, 1990).

Secretin is a bioactive peptide synthesized by upper intestinal secretin S cells (Bloom *et al.*, 1978; Chang *et al.*, 1999; Miller *et al.*, 1978; Paquette *et al.*, 1982; Strauss and Yalow, 1978). It is also synthesized in mice by the pancreas and colon (Lopez *et al.*, 1995), and by flora that inhabit the gut (Gauthier *et al.*, 2003). Whether secretin is synthesized by the forebrain is the subject of this study. Secretin belongs to the secretin/vasoactive intestinal peptide (VIP)/glucagon receptor family with actions at high and low-affinity secretin receptors (Ichihara *et al.*, 1983). It is a 27 amino acid peptide and an enterogastrone (Jin *et al.*, 1994, Li *et al.*, 1998). Secretin receptors couple to G proteins that stimulate adenylate cyclase and, in turn, lead to the production of cyclic adenosine monophosphate (cAMP) and the stimulation of associated second messenger systems (Harmar, 2001). Secretin receptors concentrate in brain regions (Itoh *et al.*, 1991, Ohta *et al.*, 1992) that are responsive to intracerebroventricular (i.c.v.) administration of the secretin peptide (Welch *et al.*, 2002a,b, 2003a). These regions are also sites of pathology in autism (Bauman and Kemper 1985, Haznedar *et al.*, 2000, Ogai *et al.*, 2003, Schultz *et al.*, 2003).

Secretin's peripheral role as a gastric hormone has long been established (Bayliss and Starling 1902); less is known about its central actions. Secretin regulates the central and peripheral stress axes via neurohumoral mechanisms (Ruggiero *et al.*, 2003, Welch *et al.*, 2002a,b, 2003a,b) that involve interactions with other signaling systems acting at the level of the hypothalamus, such as secretin/angiotensin (Walker *et al.*, 1999) and secretin/dopamine (Fuxe *et al.*, 1979). Secretin functions to modulate HPA stress axis output, and, in contrast to VIP, increases norepinephrine and dopamine turnover in the hypothalamus and median eminence (Fuxe *et al.*, 1979). Assays finding positive secretin bioactivity, radioimmunoactivity, or secretin precursor mRNA expression indicated high secretin levels in the hypothalamus and hypophysis, with the preponderance of evidence suggesting that the hypothalamus is a site of origin of endogenous secretin (Chang *et al.*, 1985; Charleton *et al.*, 1981; Fuxe *et al.*, 1979; Itoh *et al.*, 1991; Mutt *et al.*, 1979; Nussdorfer *et al.*, 2000; O'Donohue *et al.*, 1981; Ohta *et al.*, 1992; Samson *et al.*, 1984). Another study assessing the presence of secretin in the rat brain and gut did not find central expression of the bioactive peptide (Kopin *et al.*, 1990). These studies, however, lacked the single-cell resolution needed to precisely

delineate the organization of a presumptive secretinergetic system. Recently, studies found secretin immunoreactivity in the brain stem and cerebellum (Koves *et al.*, 2002; Ng *et al.*, 2002; Yung *et al.*, 2001), but not in the forebrain. According to Ng, "secretin is only present at detectable levels in the brain stem and cerebellum," although unpublished data suggest "the presence of secretin-producing cells in the cerebral cortex" (Ng *et al.*, 2002).

Prior studies led our laboratory to test the hypothesis that secretin regulates stress response patterns via endogenous synthesis along the hypothalamic stress axis. Central secretin administration (i.c.v.) activates the area postrema, nucleus of the solitary tract (NTS), and its terminal fields, including the parabrachial complex, amygdala, and hypothalamus. In addition, secretin activates the visceral thalamus and its insula/orbital and medial prefrontal cortical projection fields, which regulate visceral reflex networks overlapping areas of pathology in autism (Welch *et al.*, 2002a,b, 2003a). Corroborating some of these results was a report focused on the effects of secretin-induced *c-fos* gene expression in the amygdala of rats (Goulet *et al.*, 2003). In a subsequent study, long-term systemic administration of bioactive peptides, including trials with secretin, was found to resolve inflammatory bowel lesions and stress-related effects on specific CNS regions in mice and rats that correspond to sites of pathology in autism (Welch *et al.*, 2003b). Systemic exogenous secretin was found to reestablish communicative and affiliative interactions in autistic children with gastrointestinal abnormalities (Horvath *et al.*, 1998), an observation supported in a subsequent case report (Lamson and Plaza, 2001). This interest in secretin has led to a current investigative effort to replicate Horvath's novel peptide therapy (Wheeler 2003).

To date, there has been no study with single-cell resolution identifying secretinergetic neurons in the forebrain. In this study, we sought to determine whether secretin is synthesized specifically in the forebrain, and whether its specificity and distribution patterns might predict possible interactions of secretin with other hormones involved in stress adaptation. Additionally, we investigated whether secretin is synthesized on demand in distinct areas of the HPA stress axis of rats. The central distribution of secretin immunoreactivity in adult male Sprague–Dawley rats was mapped to single-cell resolution using immunocytochemistry. To demonstrate secretin's specificity, the experiments included preadsorption controls, as well as studies cross-comparing the distribution of secretin and other members of its peptide family, including VIP, glucagon, and pituitary-adenylate-cyclase-activating peptide (PACAP).

## METHODS

This study was approved by the *Columbia University Institutional Animal Care and Use Committee*. Experiments were performed on 12 male Sprague–Dawley rats (250–300 g) housed in the New York State Psychiatric Institute animal facility and maintained in a thermally controlled, light-cycled environment with lab chow and water ad libitum. The experiments were performed according to NIH guidelines.

Colchicine pretreatment was necessary to identify secretin immunoreactivity product in neurons expressing constitutively low basal secretin immunolabeling as