

## Secretin Activates Visceral Brain Regions in the Rat Including Areas Abnormal in Autism

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Received July 15, 2002; accepted October 1, 2002

### SUMMARY

1. The aim of this study was to determine whether central networks are involved in the presumptive behavioral and autonomic regulatory actions of secretin, a gut hormone that has been reported to have ameliorative effects in autistic children.

2. Central neural responses monitored by regional *c-fos* gene expression were examined in response to intracerebroventricular secretin injection in awake, freely-moving Sprague-Dawley rats. Tissue sections were incubated in an antibody to the *c-fos* gene product, Fos, and processed immunohistochemically.

3. Qualitative differences in Fos immunoreactivity in stress adaptation and visceral representation areas of the brain were observed between secretin- and vehicle-infused age-matched pairs ( $n = 4$  pairs). Secretin-activated regions include the area postrema, dorsal motor nucleus, medial region of the nucleus of the solitary tract and its relay station in the lateral tegmentum, locus ceruleus, ventral periaqueductal gray, periventricular thalamic nucleus, paraventricular hypothalamus magnocellularis, medial and central amygdala, lateral septal complex as well as ependymal and subependymal nuclei lining the third ventricle. Specific areas of the cerebral cortex were heavily labeled in secretin-treated rats, as compared to controls: the medial bank of the anterior prefrontal cortex, orbitofrontal cortex, the piriform cortex, and the anterior olfactory nucleus. Secretin attenuated Fos immunoreactivity in the dorsal periaqueductal gray, intralaminar thalamus, medial parvocellular compartment of the hypothalamus, supraoptic nucleus of the hypothalamus, lateral amygdala, motor cortex, and the somatosensory and association areas of the parietal cortex.

4. Secretin alters the activity of structures involved in behavioral conditioning of stress adaptation and visceral reflex reactions. This study predicts a possible cellular mechanism, activation of third ventricular ependymal and subependymal cells, as well as central regulatory actions of secretin. The physiological effects of secretin on behavioral, endocrine, autonomic and sensory neuronal activation patterns, together, contribute to central *c-fos* activation. Secretin alters the activity of structures involved in behavioral conditioning of stress adaptation and visceral reflex reactions. This study predicts a possible cellular

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mechanism, activation of third ventricular ependymal and subependymal cells, and central regulatory actions of secretin. The physiological effects of secretin on behavioral, endocrine, autonomic and sensory neuronal activation patterns, together, contribute to central *c-fos* activation. These findings mandate further investigation of secretin as a brain/gut stress regulatory hormone.

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**KEY WORDS:** secretin; autism; stress; *c-fos*; ependyma; visceral brain; stress adaptation networks.

## INTRODUCTION

A natural, endogenous form of the brain/gut hormone secretin, a basic polypeptide composed of 27 amino acids, has been suggested to ameliorate autistic behaviors and improve social interactions and expressive language in a clinical case study of boys undergoing routine endoscopy for inflammatory bowel disease (Horvath *et al.*, 1998). In a later report, transdermal administration of secretin, given daily to an autistic child, was associated with dramatic speech and developmental and behavioral gains, which were lost immediately upon discontinuation of secretin and restored within 24 h of further dosing (Lamson and Plaza, 2001). Since the ameliorations were observed independently by four families, the serendipitous discoveries were compelling, though open to question, given the failures of several other studies to corroborate the observations (Coniglio *et al.*, 2001; Dunn-Geier *et al.*, 2000; Lightdale *et al.*, 2001; Owley *et al.*, 2001; Roberts *et al.*, 2001; Robinson, 2001; Sandler *et al.*, 1999).

Although secretin has been identified primarily as a gut hormone, it has been shown to have regulatory effects on other organ systems, including the immune system, the central nervous system, the endocrine system, and the respiratory and cardiovascular systems. Van Tol *et al.* (1991) found that secretin plays a role in the regulation of cellular cytotoxicity against tumor cells. Several studies have reported secretin immunoreactivity in widespread areas of the central nervous system (Chang *et al.*, 1985; Mutt *et al.*, 1979; O'Donohue *et al.*, 1981). Many of the studies have focused particularly on the hypothalamus of various species (Chang *et al.*, 1985; Charlton *et al.*, 1981; Mutt *et al.*, 1979; O'Donohue *et al.*, 1981; Samson *et al.*, 1984). Though Kopin *et al.* (1990) failed to detect secretin mRNA in the central nervous system (CNS), Itoh *et al.* (1991) and Ohta *et al.* (1992) reported CNS expression of an mRNA precursor to secretin in the brainstem, thalamus, hypothalamus, and cerebral cortex. In a later study by Nozaki *et al.* (2002) [<sup>125</sup>I] secretin was found to bind with specificity and high affinity to the nucleus of the solitary tract, and other regions in the brainstem, thalamus, hypothalamus, and cerebral cortex.

Secretin has long been thought to be a central neuromodulator, prompting several studies examining the actions of secretin injected into the cerebroventricular system: Charlton *et al.* (1981) found that intracerebroventricular (i.c.v.) injection of secretin in rats significantly increased defecation, decreased novel-object approaches and open-field locomotor activity, and altered respiration. Weick *et al.* (1992) showed that i.c.v. secretin administration inhibited pulsatile luteinizing hormone secretion in the ovariectomized rat. It is conceivable that secretin is synthesized endogenously in the central nervous system (Fuxe *et al.*, 1979; Itoh *et al.*, 1991; O'Donohue *et al.*, 1981; Ohta *et al.*, 1992).

In the cardiovascular system, Gunnes *et al.* (1983) found in human subjects that secretin has both ionotropic and vasodilating effects. Kitani *et al.* (1978) found that secretin increased cardiac output distribution to the stomach, small intestine, and pancreas in rats. In another study, i.c.v. secretin injection induced hypothermia and elevated blood pressure without effects on heart rate (Shido *et al.* 1989), although the central sites of action were not identified.

Taken together, these data suggest that secretin is a regulatory hormone with peripheral and central mechanisms of action on multiple organ systems. Although one hypothesized mechanism of secretin is its well-established role in regulating gut function, secretin's regulatory role in other organ systems may underlie some of its ameliorative, though short-lived, actions in autistic children (Horvath *et al.*, 1998). Earlier studies have localized secretin and its presumptive receptor binding sites in viscerolimbic brain regions involved in central autonomic regulation (Itoh *et al.*, 1991; Nozaki *et al.*, 2002; Ohta *et al.*, 1992). To date, no study has examined the effects of i.c.v. administration of secretin on visceral brain regions that may be differentially activated.

This study was designed to determine whether central networks are involved in the presumptive behavioral and autonomic regulatory actions of secretin by examining the regional distribution of *c-fos* gene protein expression in response to secretin injection into the lateral ventricle of freely moving male Sprague-Dawley rats. Our approach to identifying functional neural networks activated by i.c.v. secretin administration is to use immediate early gene transcriptional regulators to monitor neuronal response patterns (Dragunow and Faull, 1989). Immediate early genes are transiently expressed in active neurons that are stimulated by cytokines, growth factors or neuropeptides, providing a powerful tool for defining neuropeptidergic pathways (Strand, 1999). The *c-fos* gene is a member of the family of immediate early response genes that are expressed in response to a wide variety of peripheral and central stimuli (Lovick, 1997; Sica *et al.*, 1999, 2000a,b; Takemoto *et al.*, 1995; Vaccarino *et al.*, 1992). Immunohistochemical staining for the *c-fos* protein, Fos, has been used to map the neuroanatomical substrates for physiological response patterns and has been used as an effective means of identifying neurons that are activated by a specific physiological stimulus (Li and Dampney, 1992).

The specific regional cerebral activity patterns reported in this study suggest a role of secretin in central neuronal function related to behavioral, endocrine, and autonomic regulation. The data, which suggest a neuroanatomical basis for the effects of secretin in autistic children, were reported in abstract form (Welch *et al.*, 2002).

## MATERIALS AND METHODS

### Animals and Surgical Procedures

Data were obtained in eight adult male Sprague-Dawley rats weighing 250–450 g. Rats were obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA) and housed at the New York State Psychiatric Institute Housing Facility. This study was approved by the Columbia University Institutional Animal Care and Utilization Committee.