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Secretin as a Neuropeptide

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Abstract

The role of secretin as a classical hormone in the gastrointestinal system is well-established. The recent debate on the use of secretin as a potential therapeutic treatment for autistic patients urges a better understanding of the neuroactive functions of secretin. Indeed, there is an increasing body of evidence pointing to the direction that, in addition to other peptides in the secretin/glucagon superfamily, secretin is also a neuropeptide. The purpose of this review is to discuss the recent data for supporting the neurocrine roles of secretin in rodents. By *in situ* hybridization and immunostaining, secretin was found to be expressed in distinct neuronal populations within the cerebellum and cerebral cortex, whereas the receptor transcript was found throughout the brain. In the rat cerebellum, secretin functions as a retrograde messenger to facilitate GABA transmission, indicating that it can modulate motor and other functions. In summary, the recent data support strongly the neuropeptide role of secretin, although the secretin-autism link remains to be clarified in the future.

Index Entries: Secretin; neuropeptide; autism; cerebellum; Purkinje cells; GABA; inhibitory postsynaptic currents.

Introduction

Secretin was the first hormone identified in history. The discovery of secretin dates back to a hundred years ago when scientists began to investigate the mechanism for pancreatic secretion in dogs. They found that hydrochloric acid in the proximal small intestine was the most potent physiological stimulant for the secretion of pancreatic juice. However, hydrochloric acid in the circulating blood had no effect on pancreatic secretion. Later, in a series of experiments conducted between 1902 and 1904, Bayliss and Starling substantiated the results of previous

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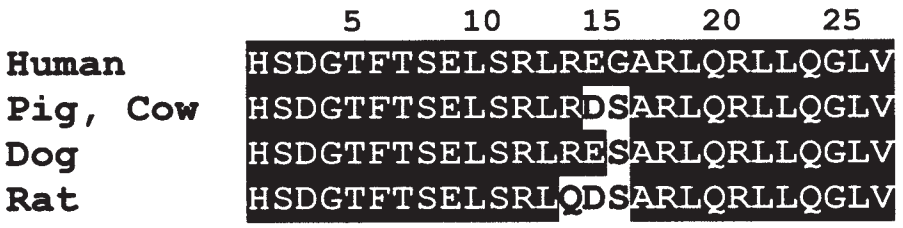

Fig. 1. Evolutionary conservation of secretin. Amino acid sequences of secretin in various mammalian species are compared. Amino acid residues identical to those of human secretin are highlighted in black. Secretin has been highly conserved throughout mammalian evolution with amino acid substitutions limited to positions 14, 15, and 16 only.

studies by showing that intravenous injection of a mucosal extract from the duodenum resulted in copious release of bicarbonate and water from the pancreas *(1,2).* They proposed that, in the duodenum, the stimulatory effect of acid in pancreatic secretion was due to the release of a messenger in the upper intestinal mucosa that entered the blood stream and subsequently exerted its excitatory effect on the pancreas. They named this blood-borne factor "secretin." Since then, a chemical messenger functionally analogous to secretin was called a "hormone," which means "to excite" in Greek *(3,4).*

Secretin was first purified from the porcine intestine by Jorpes and Mutt *(5).* The amino acid sequence of secretin was later determined by the same group, and it was found to be a basic 27-amino acid peptide *(6).* A higher molecular-weight peptide with secretin-like activity has also been identified to be a precursor of the hormone *(7,8).* Secretin has been isolated and sequenced, in chronological order, from the pig *(6),* chicken *(9),* cow *(10),* human *(11),* dog *(12),* and rat *(13).* Secretin has been conserved throughout mammalian evolution with amino acid substitutions limited to positions 14, 15, and 16 only (Fig. 1). Amino acid sequence alignment of secretin with other brain-gut peptides revealed that it belongs to a family of brain-gut peptides including vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptide (PACAP), growth hormone-releasing hormone (GHRH), peptide histidine isoleucine (PHI) or peptide histidine methionine (PHM), glucagon, glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2, and gastric inhibitory peptide (GIP) *(14).* This family of peptides has been termed the secretin/glucagon/VIP superfamily and the structural similarity between these peptides is located at the N-terminus (Fig. 2).

The principal function of secretin is to stimulate the secretion of bicarbonate, water, and electrolytes from the pancreatic ductal epithelium in response to gastric acid and fatty acids present in the duodenum *(15,16).* The released bicarbonate is important for neutralizing the acidic chyme from the stomach, thereby providing an optimal pH for the normal functioning of digestive enzymes in the small intestine. Moreover, in combination with the mucus and bicarbonate ions secreted from the epithelial lining of the gut, the pancreatic secretion forms a protective alkaline layer to prevent duodenal ulcer *(17).* Secretin also potentiates the effect of cholecystokinin in stimulating enzyme secretion from the pancreatic acinar cells *(18)* and promotes pancreatic growth *(19–21).*

Apart from the pancreas, secretin is also involved in other gastrointestinal functions, with target tissues widely distributed in our body. In the stomach, it acts as an enterogastrone that inhibits gastric acid release and gastric emptying *(22–26).* In the gall bladder, it stimulates bile flow and increases bicarbonate concentration of bile *(27).* In the duodenum, it facilitates the secretion of mucus, bicarbonate, and epidermal growth factor (EGF) from Brunner's gland *(28).* In the kidney, it regulates urine output and activates adenylyl cyclase in rats *(29).* In the

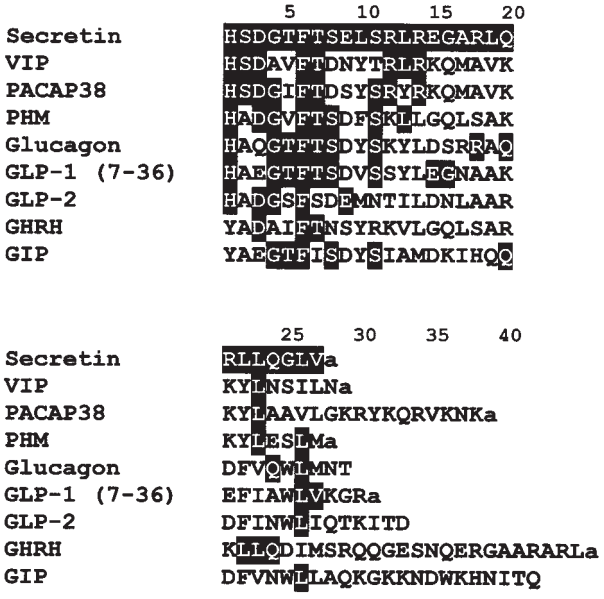

Fig. 2. Amino acid sequence alignment of human secretin with other human peptides in the same family. Residues identical to those of human secretin are highlighted in black, and the presence of C-terminal α-amidation is shown as "a." Notice that the structural similarity between these peptides is located at the N-terminus.

heart, it is a potent stimulant for contraction and cAMP accumulation in cardiomyocytes *(30).*

Although the gastrointestinal functions of secretin are well-established, its neuroactive role has been speculative. The recent discovery of the potential therapeutic use of secretin in treating autistic patients *(31,32),* together with the conflicting reports on its effectiveness *(33–40),* has raised the question on whether secretin acts in the central nervous system (CNS). The aim of this article is to review the recent evidence for supporting the role of secretin as a neuropeptide.

Functional Evidence

The function of secretin in the brain has been a subject of much speculation, although there is evidence indicating that secretin is neuroactive in the central and peripheral nervous systems. In the rat, intracerebralventricular injection of secretin results in physiological, metabolic, and behavioral changes, including stimulation of hypothalamic tyrosine hydroxylase activity *(41),* dopamine metabolism *(42),* and defecation *(43),* but inhibition of prolactin release *(41,44),* respiration, open-field activity, and novel item approach *(43).* In superior cervical ganglion and PC-12 pheochromocytoma cells, secretin stimulates tyrosine hydroxylase activity *(45,46).* Moreover, secretin stimulates cAMP accumulation in brain slices *(47),* cultured brain cells *(48),* as well as neuroblastoma-glioma hybrid cells *(49).* Taken together, these data suggest that secretin may act as a neuropeptide in the brain, possibly via a cAMP-dependent mechanism.

Recently, secretin has aroused considerable research interest, primarily attributed to the discovery of its potential therapeutic effects on autistic patients. Autism, first described by Leo Kanner in 1943, is a profound, poorly understood neurodevelopmental disorder characterized by impaired social skills, delayed language development, and stereotyped behavior. It usually affects children before 3 years of age and it occurs in approx 1 of every 2,000 live births. Children and adults with autism are normal in appearance and physically well-developed. However, their most distinctive feature, which helps us to distinguish them from those solely mentally retarded, is that they seem isolated or detached from the world around them. Very often, they also display other common symptoms, such as repeated body movements *(50),* chronic gastrointestinal problems *(51),* and sleep disturbances *(52,53).* Because autistic patients show a combination of symptoms that are rarely the same from one individual to another, several autistic subtypes have been described and the term "autistic spectrum disorder" (ASD) or "pervasive developmental disorder" (PDD) is frequently used to acknowledge the diversity of autistic symptoms (for a review, *see* ref. *54*).

The secretin-autism connection was discovered in 1998 by Victoria Beck, who is a mother

of an autistic child. At that time, her son was a 3-yr-old boy who displayed all the core symptoms of autism. Similar to some autistic children, he also suffered from chronic gastrointestinal problems like diarrhea and constipation. In a routine medical checkup, secretin was injected intravenously to assess his pancreatic functions. What surprised his parents was that shortly after the injection, his autistic symptoms lessened dramatically. He began to speak simple words, make eye contact, and have normal bowel movements. Soon after the release of Beck's anecdotal report, information spread rapidly over the internet and many parents of the autistic children requested off-label trials for the secretin treatment. At the same time, randomized, placebo-controlled trials for secretin began. The first controlled trial was done in the same year by Horvath et al., in which three children with autistic spectrum disorder were treated with secretin *(31).* Within 5 wk of the secretin infusion, a significant alleviation of the gastrointestinal symptoms was observed. Also, there was dramatic improvement in their behavior, manifested by improved eye contact, alertness, and language ability. However, following this study, conflicting reports from other controlled and randomized trials have emerged stating that secretin, either in a single-dose or repeated doses, has not shown efficacy when compared to the placebo *(33–40).* Given the heterogeneity in the pathogenesis of autism, these contradictory reports suggest that a subset of autistic children could respond to secretin, but such a conclusion will require further controlled clinical trials.

The mechanism by which intravenous secretin infusion exerts its effects on the autistic patients is largely unknown. Nonetheless, several hypotheses have been put forward. One of these hypotheses states that secretin, as a gastrointestinal hormone, may help to normalize the gastrointestinal functions in the autistic children or even improve the ability of the gastrointestinal tract to eliminate compounds that are detrimental to the brain. Another hypothesis is that, as a putative neuropeptide, secretin may directly regulate brain functions *(31).*

Evidence from Expression and Cellular Localization of Secretin and its Receptor in the CNS

To act as a neuropeptide, secretin must be synthesized in specific regions in the nervous system, where it may function as a neurotransmitter or neuromodulator. Apart from the gastrointestinal tract, the presence of secretin and secretin receptors in other tissues, especially in the CNS, is controversial. First, there are contradictory reports indicating their presence *(55,56)* or absence *(57,58)* in the brain. Second, within the CNS, no physiological action has been ascribed to secretin yet. Third, although secretin-like immunoreactivity has been detected in mammalian brain extracts *(59,60),* the identification of secretin immunoreactive cells in the brain could be complicated by potential cross-reactivity of secretin antibodies with other structurally related peptides like PACAP and VIP. Nevertheless, molecular cloning of secretin and secretin receptor cDNAs has facilitated the use of sensitive hybridization techniques to detect the presence of secretin and its receptor in the brain. For example, using reverse transcriptase-polymerase chain reaction (RT-PCR), secretin transcripts have been detected in specific regions of the rat brain such as hypothalamus, brainstem, cortex, thalamus, hippocampus, and medulla oblongata *(55,56).* Moreover, Northern blot analysis indicated that secretin mRNA is expressed at low levels at most brain regions *(61).* As a whole, these data indicate that secretin is expressed, albeit at relatively low levels, throughout the CNS. In contrast, the findings regarding the expression of secretin receptors in the brain are more divergent. Using [^{125}I]-labeled secretin, high-affinity binding sites to the rat brain membranes were detected *(62).* On the other hand, using Northern blot analysis, secretin receptor mRNA has not been detected in the whole brain *(58,63),* suggesting that secretin receptors are either expressed at low levels below the detection limit of Northern blot, or localized only in specific regions of the brain. However, secretin receptors

have not been detected in various regions of the brain, nor has there been any direct histochemical evidence to indicate that secretin and secretin receptors are expressed in central neurons.

For these reasons, the expression of secretin and its receptor in various regions of the rat brain, including the cortex, hippocampus, hypothalamus, brainstem, striatum, and thalamus was investigated using Northern blot analysis (Fig. 3). We found that secretin receptors are expressed in all the brain regions studied. However, secretin is only present at detectable levels in the brainstem and cerebellum. Although some brain regions do not show expression of secretin within the detection limits of Northern blotting, it is possible that secretin may be locally expressed in small populations of distinct neuronal cells. For example, our immunostaining data in the rat brain also showed the presence of secretin-producing cells in the cerebral cortex (unpublished data). The presence of secretin receptor transcript throughout the brain suggests that many neuronal functions could be modulated by secretin produced and released locally or via axonal networks from other parts of the brain. In addition, the neural effects of secretin could also be originated from the gastrointestinal tract via the blood stream. To this end, blood-borne secretin may either have vagal actions on the CNS or it may be able to pass through the blood-brain barrier (BBB), which allows small or lipophilic molecules to enter the brain. Whether or not secretin, being a water-soluble peptide, crosses the BBB remains to be clarified. However, the existence of a bidirectional relationship between secretin in the brain and that in the gut has been supported by the observations that: 1) intracerebralventricular injection of secretin-stimulated pancreatic volume and bicarbonate release in rats *(64);* 2) intravenous injection and transdermal administration of secretin led to dramatic improvements in the cognitive functions of some autistic patients *(31,32);* 3) a fully functional radioiodinated secretin analog ([tyr^{10}]secretin) crossed the BBB from the circulation to the CNS in mice *(65);* 4) secretin infusion was able to induce c-fos mRNA and protein expression, particularly in the amygdala in rats *(66).* Thus, it appears that secretin may mediate its neuroactive functions in the brain via two routes: from locally produced secretin within the brain, and from blood-borne secretin released from the gut.

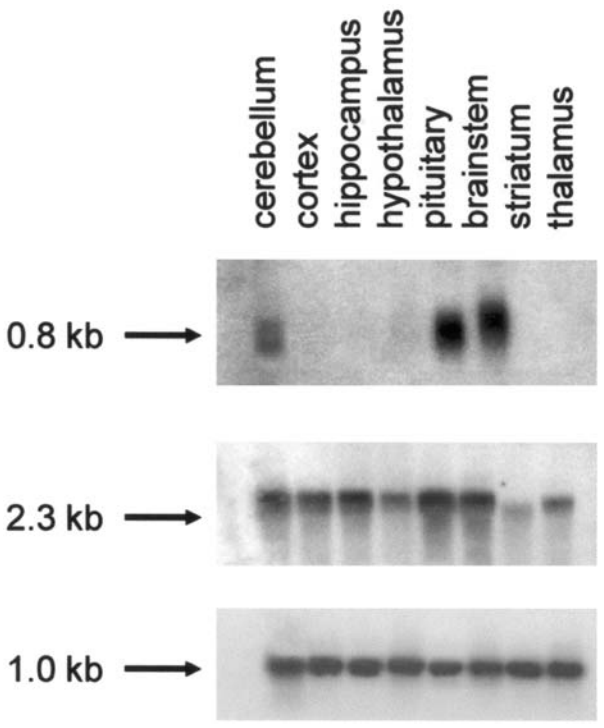

Fig. 3. Northern blot analysis of secretin (top panel) and secretin receptors (middle panel) in various regions of the rat brain. Each lane was loaded with 5 μg of Poly(A)$^+$ mRNA. Hybridization was performed using ^{32}P-labeled cDNA encoding rat secretin (453 bp; nucleotides 1-453) *(57)* or N-terminal domain of the secretin receptor (427 bp; nucleotides 213-639) *(58)*. The partial cDNAs were generated by RT-PCR using specific primers. As an internal control, the blot was re-hybridized for β-actin mRNA (bottom). The sequences of the primers for amplifying the cDNA probe for β-actin mRNA are (5′-GACGAGGCCC AGAGCAAGAGAGG-3′ and 5′-CTGCTTGCTGATCCACATCTGCT-3′) and sizes of the transcripts are indicated by arrows.

The cellular localization of secretin and its receptor in the rat cerebellum was also deter-

mined by *in situ* hybridization and immunocytochemistry *(67).* Co-localization of secretin and its receptor mRNAs was observed in the cerebellum. By *in situ* hybridization, we found that secretin and its receptor are expressed in specific neuronal populations. Within the cerebellar cortex, both secretin and secretin receptor transcripts are expressed in Purkinje neurons. In addition, basket cells, which are GABAergic interneurons present at the lower half of the molecular layer, were found to express secretin receptors. Expression of secretin and secretin receptors in distinct neurons strongly suggests that secretin serves specific neural functions in the cerebellum. Their distribution patterns also imply that in the cerebellar cortex, Purkinje cells synthesize secretin that is targeted onto themselves and the nearby basket cells. Consistent with the *in situ* hybridization data, secretin immunoreactivity is present in both the soma and dendrites of Purkinje cells, suggesting that, in addition to being a possible neurotransmitter, secretin may be synthesized by Purkinje cells and released at the somatodendritic regions as a neuromodulator. Within the cerebellar cortex, it is worth noting that the secretin-related peptide PACAP, synthesized by Purkinje cells, also acts as a neuromodulator *(68,69).*

Co-expression of secretin and secretin receptor transcripts were also detected in various neurons in the cerebellar deep nuclei (unpublished data). Functionally, these deep cerebellar neurons are closely related to Purkinje cells because the inhibitory output from Purkinje cells is exerted on the neurons in the cerebellar deep nuclei, which are the major output line to extracerebellar structures *(70).* Therefore, secretin may exert its neuroactive actions on extracerebellar brain areas via direct modulation on deep cerebellar neurons.

Electrophysiological Evidence in the Rat Cerebellum

A neuromodulatory role played by secretin in the CNS was directly demonstrated in the cerebellum by an electrophysiological approach *(67).* In the in vitro cerebellar slices of the rat, focal electrical stimulation within the cerebellar cortex readily evokes two types of synaptic response in voltage-clamped Purkinje cells: inhibitory postsynaptic currents (IPSCs) and excitatory postsynaptic currents (EPSCs). The IPSCs are mediated by GABA released from the terminals of basket cells and stellate cells in the molecular layer, whereas the EPSCs are due to glutamate released mainly from the parallel fibers originating from the granule cells. We found that secretin at nanomolar concentrations robustly increased the amplitudes of IPSCs but not that of the EPSCs. Thus, GABAergic transmission onto the Purkinje cells is selectively facilitated by secretin. Although both basket cells and Purkinje cells express secretin receptor transcripts, no acute postsynaptic effect was observed in these cells when secretin was applied. Further experiments on tetrodotoxin-resistant miniature IPSCs confirmed that secretin facilitates vesicular release of GABA by acting at a presynaptic site, most likely on the axon terminals of the basket cells. Based on the relatively high potency of secretin (EC_{50} ~14 n*M*) and the lack of effect of VIP and PACAP on the IPSCs, we concluded that secretin acts on secretin receptors. Similar to its effects on peripheral tissues *(22–30),* the neuromodulatory action of secretin in the presynaptic basket cells involves activation of adenylyl cyclase and a rise in intracellular cAMP. The facilitatory effect of secretin on the miniature IPSCs was, however, not dependent on extracellular calcium influx.

These electrophysiological findings of secretin in the cerebellum correlate well with many of the *in situ* hybridization and immunohistochemical observations discussed in the previous section, including the expression of secretin receptor transcript in basket cells but not in granule cells, and the presence of secretin transcript and immunoreactivity in Purkinje cells. Taken together, our findings suggest that Purkinje cells release secretin at the somato-dendritic region and stabilize themselves by facilitating the inhibitory inputs from the basket cells (*see* Fig. 4 for a model). Furthermore, the cerebellum is enriched with a

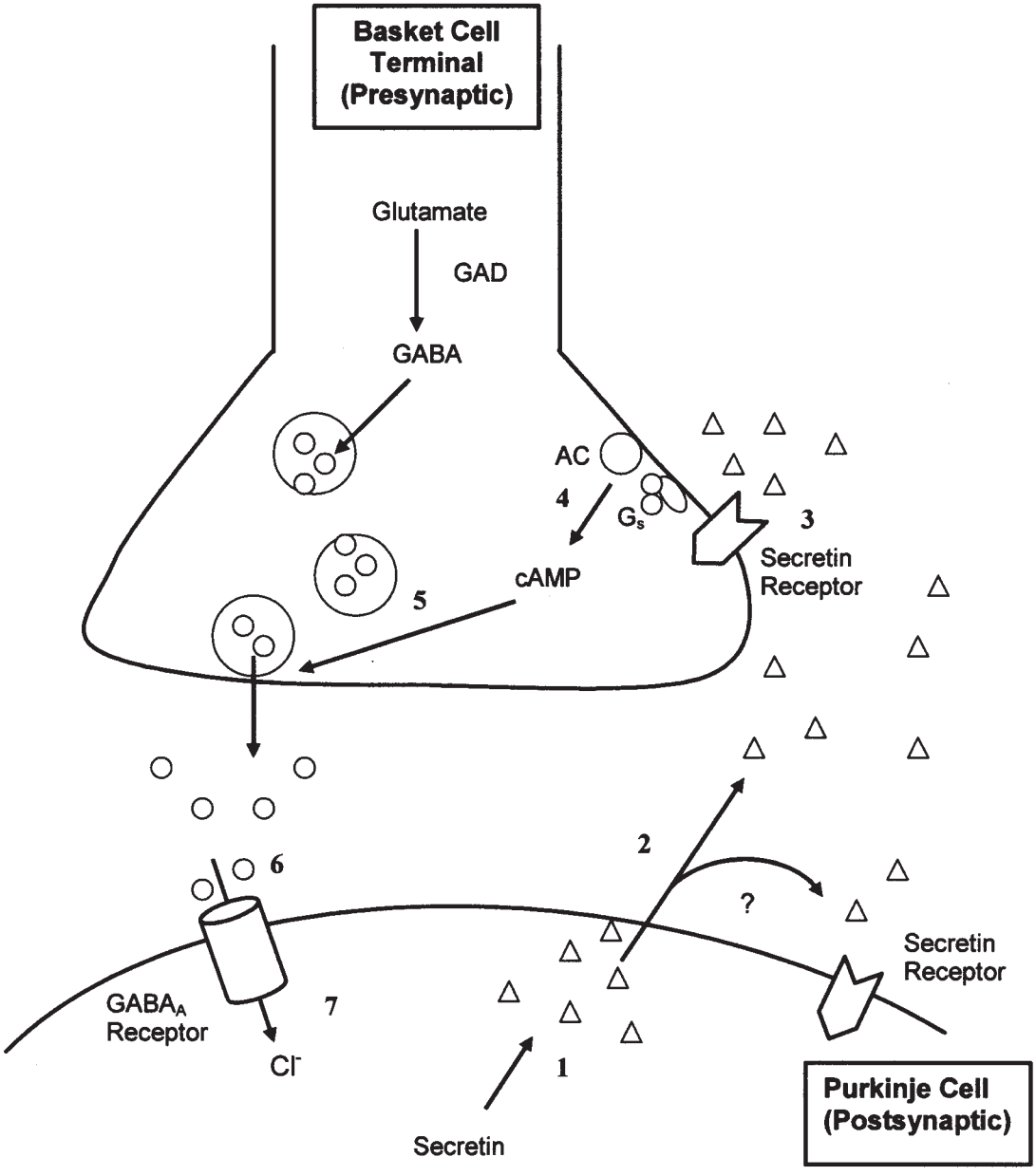

Fig. 4. Diagrammatic representation of a proposed model for the neuroactive function of secretin in the cerebellum. The numbers indicate the steps for the facilitatory effect of secretin in GABA transmission: 1) Secretin is synthesized in the soma and transported to the dendrites of Purkinje cells; 2) Secretin is released from the somatodendritic region of Purkinje cells; 3) Secretin binds to secretin receptors on the presynaptic membrane or on the Purkinje cell membrane; 4) Secretin activates adenylyl cyclase and increases the cAMP level in the presynaptic basket cell; 5) The increased cAMP level facilitates GABA release from the basket cell; 6) GABA binds to ionotropic $GABA_A$ receptors on the Purkinje cell membrane; 7) Chloride ions enter Purkinje cells, leading to the generation of IPSCs. In this model, the endogenous signal for triggering secretin release and the functions of secretin receptors in Purkinje cells remain unclear. Abbreviations: AC, adenylyl cyclase; Gs, stimulatory G protein; GAD, glutamic acid decarboxylase.

set of distinct plastic phenomena including long-term depression, depolarization-induced suppression of inhibition, and depolarization-induced rebound facilitation *(71).* Because the effect of secretin is long-lasting, it may be involved in some of these phenomena that have been implicated in motor learning and other functions of the cerebellum *(71,72).*

Conclusions and Future Directions

In this review, using rodents as animal models, we have provided evidence in favor of a neuroactive role of secretin in the CNS, especially in the cerebellar cortex. To further establish that secretin is a neuropeptide in the cerebellum, we need to: 1) provide evidence for the endogenous stimulation and release of secretin under various physiological conditions; 2) show that the released secretin molecules are able to diffuse and interact with specific cell-surface receptors on neurons in the vicinity; 3) elaborate the post-receptor mechanisms for neuronal activation. The working model of secretin as a retrograde messenger for the stabilization of Purkinje cells remains to be scrutinized. One unanswered question in this working model is what the functions of secretin receptors expressed on the cell surface of Purkinje cells are. As secretin is also expressed in the deep cerebellar nuclei (unpublished data), it is likely that secretin released from deep cerebellar neurons may also act as a retrograde messenger to potentiate GABA release from Purkinje cells. It is therefore possible that secretin functions as a retrograde messenger at multiple levels within the complicated neuronal networks of the cerebellum to modulate motor and other functions of the rat cerebellum.

What about the presence of secretin and secretin receptor in other parts of the rat brain? It seems that we still have a long way to uncover the full potential of the neurocrine functions of secretin. To fully understand the neurophysiological role of secretin, we need tools such as specific antibodies for the receptor, functional antagonists with known mechanisms of inhibition, and most importantly, cell-targeted knockout mice models for secretin and its receptor.

The expression and direct electrophysiological action of secretin in rat cerebellum are established; unfortunately, there is no convincing evidence as yet to show that secretin and secretin receptors are expressed in distinct cells within the CNS of humans. As indicated by the higher concordance rate of autism in monozygotic (60%) than that in dizygotic (0%) twins *(73),* there is obviously a genetic basis for this disease. The most well-established candidate loci for autism are 2q, 7q, 15q11-13, and 16p *(74,75),* whereas the human secretin and secretin receptor genes are mapped to 11p15.5 *(76)* and 2q14 *(77),* respectively. Although the chromosome localization of the human secretin receptor gene matches one of these candidate loci, as yet, there is no convincing evidence to indicate a clear genetic linkage between autism and mutations of secretin and/or secretin receptor genes.

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