Neuroscience 134 (2005) 377–386

ENDOGENOUS RELEASE AND MULTIPLE ACTIONS OF SECRETIN IN THE RAT CEREBELLUM

S. M. Y. LEE,[a] L. CHEN,[b] B. K. C. CHOW[a] AND W. H. YUNG[b]*

[a]*Department of Zoology, The University of Hong Kong, Pokfulam, Hong Kong, China*

[b]*Department of Physiology, Faculty of Medicine, the Chinese University of Hong Kong, Shatin, Hong Kong, China*

Abstract—Previous studies demonstrated that secretin could modulate synaptic transmission in the rat cerebellum. In the present report, we provide evidence for the endogenous release of secretin in the cerebellum and further characterize the actions of secretin in this brain area. First, to show that secretin is released endogenously, blocks of freshly dissected cerebella were challenged with a high concentration of KCl. Incubation with KCl almost doubled the rate of secretin release. This KCl-induced release was sensitive to tetrodotoxin and cadmium suggesting the involvement of voltage-gated sodium and calcium channels. The use of specific channel blockers further revealed that L-type and P/Q-type calcium channels underlie both basal and KCl-evoked secretin release. In support of this, depolarization of Purkinje neurons in the presence of NMDA, group II mGluR and cannabinoid CB1 receptor blockers resulted in increased inhibitory postsynaptic current frequency. Second, we found that the previously reported facilitatory action of secretin on GABAergic inputs to Purkinje neurons is partly dependent on the release of endogenous glutamate. In the presence of CNQX, an AMPA/kainate receptor antagonist, the facilitatory effect of secretin on GABA release was significantly reduced. In support of this idea, application of AMPA, but not kainate receptor agonist, facilitated GABA release from inhibitory terminals, an action that was sensitive to AMPA receptor antagonists. These data indicate that a direct and an indirect pathway mediate the action of secretin in the basket cell–Purkinje neuron synapse. The results provide further and more solid evidence for the role of secretin as a neuropeptide in the mammalian CNS. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: secretin, neuropeptide, cerebellum, glutamate.

Secretin belongs to a family of gut–brain peptides that also include glucagon, vasoactive intestinal polypeptide, pituitary adenylate cyclase-activating peptide and other structurally related peptides. By interacting with specific G-protein-coupled receptor, namely secretin receptor, one of the main functions of secretin is to regulate the secretion of pancreatic bicarbonate, water and electrolytes (Chey and Chang, 2003). In recent years, molecular approaches are beginning to unravel the organization and transcriptional regulation of secretin and secretin receptor genes, including that of human (Ho et al., 1999; Yamagata et al., 2002; Lee et al., 2004).

The discovery that secretin injection could ameliorate symptoms of autistic children (Horvath et al., 1988) started a debate on the therapeutic value of this peptide that has continued up to present (Kern et al., 2004 for a review). Despite such a controversy, there is evidence accumulated in past few years showing that secretin and its receptors are expressed in CNS and that secretin is neuroactive (Ng et al., 2002; Goulet et al., 2003; Welch et al., 2003, 2004). In mammalian brain, areas found to be affected by secretin administration include cerebellum (Yung et al., 2001), brain stem (Welch et al., 2003; Yang et al., 2004), amygdala (Goulet et al., 2003), hippocampus (Kuntz et al., 2004) and cortex (Welch et al., 2003). Our laboratory previously reported that secretin selectively facilitates release of GABA from basket cell terminals in the cerebellum via, partly, the cAMP-PKA pathway. Together with our finding that secretin and its receptor are expressed on distinct neuronal populations we hypothesized that secretin is a neuropeptide in cerebellum serving as a retrograde messenger (Yung et al., 2001). To confirm this hypothesis, however, it is essential to demonstrate that secretin is released endogenously from cerebellum. To address this question, in this study, we tried to evoke release of secretin from cerebellum, and measured by enzyme immunoassay technique. In addition to the issue of endogenous release, there are important questions about the action of secretin in cerebellum that need to be clarified. Notably, inhibitors of cAMP-PKA pathway do not completely block the action of secretin, suggesting the involvement of additional mechanism. Here, by means of whole-cell patch-clamp recordings from Purkinje neurons we provide evidence for the unexpected finding that part of the effect of secretin on Purkinje neurons is likely to be mediated by endogenous release of glutamate acting on presynaptic AMPA receptors on basket cell terminals. These data clarify the action of secretin in cerebellum and provide further evidence that secretin is a neuropeptide in the central nervous system.

EXPERIMENTAL PROCEDURES

Sprague–Dawley rats were used for the experiments. The experimental procedures were performed in accordance to local and international guidelines on the ethical use of laboratory animals. Effort was made to minimize the number of animals used and their sufferings.

*Corresponding author. Tel: +852-26096880.
E-mail address: whyung@cuhk.edu.hk (W. H. Yung).

Abbreviations: ACSF, artificial cerebrospinal fluid; EIA, enzyme immunoassay; IPSC, inhibitory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; TTX, tetrodotoxin.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.04.009

Enzyme immunoassay

The brain of an adult rat was removed and the whole cerebellum was dissected out. It was immersed and diced into small blocks in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 2 KCl, 120 NaCl, 2 $MgSO_4$, 1.2 KH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$ and 11 glucose. The cerebellar blocks were pre-incubated in ACSF at 37 °C bubbled with carbogen for 30 min (equilibration period). They were then incubated in fresh ACSF for another 10 min for detection of basal release. To evoke depolarization-induced release of secretin, the slices were challenged with 40 mM KCl. Tetrodotoxin (TTX, 1 μM) or $CdCl_2$ (200 μM), nimodipine (40 μM) and omega-agatoxin IVA (100 nM) were used to elucidate the mechanisms of secretin release. During the course of experiments, fresh ACSF was replaced every 5 min.

To measure release of secretin, enzyme immunoassay (EIA) was performed using a commercially available EIA kit against rat secretin (Phoenix Pharmaceuticals, USA), following the procedures recommended by the supplier. After 30-min equilibration period, samples were collected every 5 min to establish the basal release as well as to monitor changes in release due to different drug treatments. Briefly, 50 μl of a standard or a sample, 25 μl primary antiserum and 25 μl biotinylated secretin were added to wells of 96-well immunoplates. The mixture was incubated at room temperature for 2 h followed by five times of washing (300 μl/well) using assay buffer. Streptavidin–horseradish peroxidase solution was then added (100 μl/well) and incubated at room temperature for 1 h, followed by six times of washing. Substrate solution (100 μl/well) was then added and incubated at room temperature for 1 h. Finally, the reaction was terminated by adding 100 μl of 2 N HCl to each well. Optical density readings were obtained at 450 nm and the amounts of secretin present were calculated.

Electrophysiological recordings

Cerebellar slices (250 μm thick) from 12–14 days old rats were prepared by a vibrating microtome. Whole-cell patch-clamp recordings of Purkinje neurons were obtained using a conventional patch-clamp amplifier (List Electronics, Germany). A Purkinje neuron was first identified and then approached visually, aided by differential interference contrast optics and contrast-enhanced video-microscopy. Whole-cell pipettes used typically had a resistance of 3–5 MΩ when filled with an internal solution of the following composition (in mM): KCl 140, HEPES 10, EGTA 1, $MgCl_2$ 2, Na_2ATP 2, Tris GTP 0.4, and pH was adjusted to 7.25–7.30 with 1 M KOH. A giga-ohm seal was made followed by rupturing of the membrane to achieve whole-cell recording. The inclusion of 140 mM Cl^- in the internal solution enhanced the detection of GABA-A receptor mediated inhibitory postsynaptic currents (IPSCs). To trigger endogenous secretin release from Purkinje neurons, we employed a conditioning protocol, which consisted of 10 depolarizing pulses of 100 ms duration at 1 Hz, from a holding potential of −70 mV to 0 mV (Yoshida et al., 2002). Automatic detection and analysis of the miniature inhibitory postsynaptic currents (mIPSCs) were performed using a program developed in the laboratory or by a commercially available program (MiniAnalysis ver 5.1, SynaptoSoft).

Drugs and statistics

Drugs were obtained from the following sources: rat secretin (Bachem, Switzerland), bicuculline methiodide, CNQX, nimodipine, (RBI-Sigma, USA), AMPA, SQ22536 (Calbiochem, USA), AP5, AM251, CGP55845, EGLU, SYM2206, SYM2081 (Tocris, UK), TTX, omega-agatoxin IVA (Alomone Labs, Israel). These compounds were prepared as concentrated stocks and were diluted in ACSF to final concentrations just before use. Data are expressed as mean±S.E.M. Student's *t*-test was used to compare two groups of data.

RESULTS

Endogenous release of secretin in the cerebellum

Using the EIA method, we were able to detect release of secretin from the cerebellum. Preliminary experiments indicated that the release of secretin stabilizes after 30 min of equilibration period. In all experiments described, the basal release was measured twice, at 5 min and 10 min after the equilibration period, before challenge by drugs. Before administration of KCl, 80–200 pg/ml ($n=6$) of secretin was found to accumulate in a 5-min period. This basal release was not sensitive to TTX but was suppressed significantly by 200 μM of Cd^{2+}. When challenged with 40 mM of KCl, the rate of release of secretin increased and peaked at 10 min to a level of 194.6±4.2% of control ($n=6$, $P<0.05$). This result suggests that high KCl-induced membrane depolarization evoked release of secretin. To determine whether the activation of voltage-gated Na-channels was required for the release, the effect of TTX was studied. In the presence of 1 μM of TTX, 40 mM of KCl did not significantly increase the release of secretin (111.8±1.1%; $n=6$; $P>0.05$). Next, we tested the involvement of Ca-influx in the release of secretin. Co-application of 200 μM $CdCl_2$ also prevented a significant rise of secretin release (96.4±7.0%; $n=6$; $P>0.05$). These data, summarized in Fig. 1A, suggest that the openings of both voltage-gated Na- and Ca-channels are necessary for the evoked release of secretin while the basal release depends on Ca-channels only.

The role of L-type and P/Q-type calcium channels

Since secretin immunoreactivity is highly localized to Purkinje neurons (Ng et al., 2001; Yung et al., 2001), these cells are believed to the major source of secretin release. To study the types of Ca-channels in mediating secretin release we therefore focused on two subtypes of Ca-channels, the L-type and P/Q-type, which are known to exist at relatively high levels in Purkinje neurons (Chung et al., 2000). We found that the L-type Ca-channel blocker nimodipine (40 μM) not only inhibited KCl-induced secretin release but resulted in a rate of release less than that of basal (63.9±4.7%; $n=6$; $P<0.05$). Omega-agatoxin IVA (100 nM), a P/Q-type Ca-channel blocker, also apparently inhibited KCl-induced secretin release (94.3±6.8%; $n=6$; $P<0.05$). In addition, the basal release of secretin was sensitive to nimodipine (18.1±6.7%; $n=8$; $P<0.05$) and omega-agatoxin IVA (56.8±10.2%; $n=8$; $P<0.05$). Overall, these data suggest that both L-type and P/Q-type Ca-channels contribute to KCl-evoked release, and are consistent with the notion that they are also involved in basal release of secretin. Furthermore, L-type Ca-channels would seem to be more important in mediating secretin releasee. These data are summarized in Fig. 1B.

Depolarization of Purkinje neurons increased IPSC frequency

To provide further evidence that Purkinje neurons release secretin, we determined the effects of directly depolarizing