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Presynaptically mediated effects of cholecystokinin-8 on the excitability of area postrema neurons in rat brain slices

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ABSTRACT

Cholecystokinin (CCK) is a well-known gut hormone that shows anorexigenic effects via action at peripheral and central receptors. CCK is also widely distributed throughout the mammalian brain and appears to function as a neurotransmitter and neuromodulator. The area postrema is one of the circumventricular organs, located on the dorsal surface of the medulla oblongata at the caudal end of the fourth ventricle. Blood vessels in the area postrema lack a blood brain barrier, offering specific central neural elements unique access to circulating substances. Immunohistochemical studies show CCK-A receptors in the area postrema, and we reported CCK-sensitive area postrema neurons. However, the receptive mechanism of CCK in area postrema neurons still remains unexplained. We investigated the responses of area postrema neurons to agonists and antagonists of CCK receptors using whole cell and perforated patch-clamp recordings in rat brain slices. The application of CCK-8 elicited excitatory responses, such as increases in the frequency of mEPSCs (miniature excitatory postsynaptic currents), a shift toward larger amplitude mEPSCs, and increases in the frequency of action potentials. These changes were found mostly in cells not displaying the hyperpolarization-activated cation current (Ih), except for small excitatory changes in a minority of Ih-positive neurons. Tonic inward currents or an inhibitory response to CCK-8 were never seen. Analysis of the amplitude of mEPSCs before and after the administration of CCK-8 indicated the responses mediated via the presynaptic receptors. The effect of CCK-8 was abolished in the presence of CNQX (AMPA type

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glutamate receptor antagonist). In the presence of lorglumide (a selective CCK-A receptor antagonist), CCK-8-induced excitatory responses were inhibited. No cells responded to the administration of non-sulfated CCK-8 (CCK-8NS, a selective CCK-B receptor agonist). We conclude that CCK-8 exerts its action via presynaptic CCK-A receptors to facilitate glutamate release onto Ih-negative area postrema cells.

1. Introduction

Cholecystokinin (CCK) is originally defined as a gastrointestinal hormone that takes a facilitating role in the secretion of pancreatic digestive enzymes. Previous studies have reported that CCK inhibited feeding behavior and gastric function by acting as a paracrine modulator of vagal afferents in the periphery (Crawley and Corwin, 1994; Gibbs et al., 1973). In the central nervous system, CCK is also widely distributed throughout the mammalian brain and appears to function as a neurotransmitter and neuromodulator (Crawley and Corwin, 1994). CCK receptors can be found in the central nervous system as well as internal organs (e.g. pancreas) and the peripheral nervous system (e.g. vagus nerve terminals) (Noble et al., 1999). The suppressive effect of intraperitoneal injection of CCK on food intake was attenuated in rats with thermal lesions of the area postrema, suggesting that the area postrema may be the major site where CCK acts to decrease food intake (van der Kooy, 1984). Previous studies have demonstrated that CCK can excite neurons in the area postrema (Carpenter et al., 1988; Funahashi and Adachi, 1993; Sun and Ferguson, 1997).

Neurons in the area postrema play an important role in the regulation of feeding behavior (Edwards and Ritter, 1981; Ritter and Edwards, 1984; South and Ritter, 1983). Previous studies have shown the chemosensitivity of area postrema neurons to several kinds of intestine peptide hormones that contribute to the feeding regulation, including CCK, which is known as a hunger suppressant (Carpenter et al., 1983; Funahashi and Adachi, 1993; Sun and Ferguson, 1997). It has been shown that systemic infusion of CCK induced c-Fos expression in the area postrema, indicates that it is a receptive site for circulating CCK (Luckman et al., 1993).

As in other circumventricular organs in the brain, the area postrema neurons can access circulating chemical substances because of the lack of a blood brain barrier. We have previously demonstrated the presence of glucose responsive neurons that concurrently responded to cholecystokinin-8 (CCK-8) in the area postrema using an extracellular recording technique (Funahashi and Adachi, 1993). However the receptive mechanism of CCK-8 in area postrema neurons remains to be determined. Furthermore, our resent study demonstrated that area postrema neurons sort into two different groups based on membrane properties specified by the presence or absence of the hyperpolarization-activated cation channel (H-channel). Assessing the responsivity of area postrema cells to a peptide such as CCK necessitates the identification of which neurons respond to CCK-8.

We sought to examine the actions of CCK-8 in area postrema in greater detail about both the responsive neurons and the mechanisms of CCK action than our initial demonstration of the excitatory effects of CCK-8 (based on extracellular recording techniques; Carpenter et al., 1988; Funahashi and Adachi, 1993; Sun and Ferguson, 1997).

2. Results

Patch-clamp recordings were obtained from total 138 neurons, and CCK-8 responding neurons were found in 40 of 130 neurons recorded in conventional whole-cell mode, and in 2 of 8 neurons recorded in perforated patch mode. All responses to CCK-8 were excitatory. Cells were classified based on the presence or absence of Ih (Funahashi et al., 2002, 2003). Thirty-eight of 42 neurons that responded to CCK-8 were cells not displaying Ih; 4 of 42 responsive neurons displayed Ih.

Typical responses to CCK-8 obtained from cells not displaying Ih ($n=38/85$) are shown in Fig. 1A. In the cell shown in Fig. 1A1, the focal administration of CCK-8 elicited marked increases in the frequency of mEPSCs without inducing tonic inward currents. Fig. 1A2 shows a typical recording from a CCK-8 non-responsive Ih-negative cell. Statistical analysis showed a significant shift toward shorter inter-event intervals (Fig. 1B1, $p<0.01$ by KS test). Analysis of mEPSC amplitudes showed no significant changes in either the distribution of event amplitudes or mean amplitudes (Fig. 1B2, $p>0.05$ by KS test and $p>0.05$ by Student's t test, respectively).

In neurons displaying Ih ($n=53$), we found 4 neurons that responded to CCK-8 (Fig. 1C). The neurons showed weaker responses than Ih-negative cells. Tonic inward currents were not detected during the administration of CCK-8 in these cells. It was impossible to analyze these data with the cumulative plots and KS test because the number of mEPSCs was not enough for such analysis. In the cell shown in Fig. 1C, however, the mean frequency of mEPSCs during the application of CCK-8 was significantly increased to 218% of the control ($p<0.01$, student t test), and no significant change was detected in the mean amplitude of mEPSCs during the application of CCK-8 ($p>0.05$, Student's t test).

The effects of CCK-8 on mEPSCs were concentration-dependent as shown in Fig. 2. Typical examples are shown in Fig. 2A. Significant increases in the frequency of mEPSCs in response to CCK-8 (1, 10, 100, 500, 1000 nM, $n=2$, 3, 4, 2, 4, respectively) were supported by the cumulative probability plots that indicated a significant shift of the inter-event intervals toward shorter intervals as compared to the control (normal ACSF) ($p<0.01$, KS test, $n=200$ events in each category) (Fig. 2B1).

Plots of the amplitude distribution of mEPSCs showed a significant shift in the distribution of amplitudes elicited by