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Research Report

Electrophysiologically identified presynaptic mechanisms underlying amylinergic modulation of area postrema neuronal excitability in rat brain slices

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ABSTRACT

Amylin, which is co-secreted together with insulin by pancreatic beta cells, is considered to be an important peptide hormone involved in the control of feeding behavior and energy homeostasis. Although the area postrema has been implicated to be a primary target of amylin, there are no studies of the mechanisms by which amylin may alter the excitability of area postrema neurons. To investigate the mechanism for amylinergic modulation of neuronal excitability, we performed perforated patch-clamp recordings from area postrema neurons in rat brainstem slices. Amylin-induced changes in excitatory responses, such as increases in the frequency of mEPSCs (miniature excitatory postsynaptic currents) and changes in the amplitude distribution of mEPSCs, were found in cells not displaying the hyperpolarization-activated cation current (I_h). Area postrema cells displaying I_h did not respond to amylin application. Inhibitory responses to amylin were never encountered. Bath application of CNQX (AMPA type glutamate receptor antagonist) abolished the effects of amylin. Depolarization of cells during amylin application was sufficient at 1 μM to cause action potential discharge by responding cells. We conclude that amylin receptors are located mostly on presynaptic glutamatergic terminals connecting to the area postrema neurons not displaying I_h and amylin concentrations can increase glutamate release enough to cause cell firing. Modulation of amylinergic activity may offer a novel target to influence food intake and obesity.

1. Introduction

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 components unique access to circulating substances (Borison, 1989). A close relation between area postrema neuronal activity and autonomic functions has been suggested by many electrophysiological and behavioral studies, including the

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autonomic control of food intake (Contreras et al., 1984; van der Kooy, 1984; Ritter and Edwards, 1984), body fluid homeostasis (Miselis et al., 1984; Iovino et al., 1988), cardiovascular control (Ferguson and Smith, 1991), and emesis (Borison and Wang, 1953).

Amylin is a peptide hormone that is co-secreted with insulin from the pancreatic beta-cell in response to food intake. Previous studies have demonstrated the physiological effects of amylin, such as a reduction of food intake (Chance et al., 1991; Lutz et al., 1995b), an inhibition of gastric emptying (Young et al., 1995; Young, 2005; Clementi et al., 1996), and a suppression of glucagon secretion (Gedulin et al., 1997). The anorectic effect has attracted much attention because of the potential for obesity treatment.

Physiological and histological studies have demonstrated the multiple mechanisms for the anorectic effects of amylin. The anorectic actions of amylin occurred after peripheral administration (e.g. intraperitoneal) or central administration (e.g. intrahypothalamic or intraventricular) (Chance et al., 1991; Lutz et al., 1995b, Rushing et al., 2002). Behavioral experiments identified the area postrema as the primary site for circulating amylin since peripherally applied amylin did not show anorectic actions in area postrema-lesioned rats (Lutz et al., 1998; Barth et al., 2004). Area postrema lesions also blocked the control of gastric emptying by amylin (Edwards et al., 1998). The amylin antagonist, AC-187, injected into the area postrema blocked the anorectic effect induced by peripherally-administered amylin, furthermore, AC187 alone increased food intake in rats (Mollet et al., 2004). These studies suggest that the area postrema is the primary amylin target site, while dense amylin binding sites were found in many brain areas including the area postrema (D'Este et al., 2000; Sexton et al., 1994). Although further study is necessary to determine other target sites of amylin, the action of amylin in the area postrema is much better characterized than in any other brain regions at present.

Taking these various studies together, the anorectic effects of amylin could be mediated by the activation of a neural network from the area postrema to the rostro-dorsal lateral hypothalamic area (dLHA) through the nucleus tractus solitarius (NTS), the lateral parabrachial nucleus (LPB), the central nucleus of amygdala (Ce) and the lateral bed nucleus of stria terminalis (BSTL), i.e. AP–NTS–LPB–Ce–BSTL–dLHA axis (Becskei et al., 2007; Cline et al., 2008; Lutz et al., 1995a; Potes and Lutz, 2010; Potes et al., 2010; Riediger et al., 2004). It was also suggested that amylin-induced excitation of area postrema neurons resulted in the reduction of orexinergic neuropeptides, such as orexin and melanin concentrating hormone, in the lateral hypothalamic area (LHA) (Barth et al., 2003).

While the LHA contains neurons playing an important role in the control of feeding, it is unlikely that amylin affects LHA neurons directly, even when amylin was injected into the LHA because amylin receptors have not been detected anywhere in the LHA (Beaumont et al., 1993; Sexton et al., 1994; van Rossum et al., 1994). Rather, the effects of amylin in the hypothalamic area are more likely mediated by neurons in the satiety center and its surrounding nuclei since amylin-immunoreactive neurons have been detected in these hypothalamic nuclei, e.g. periventricular, ventromedial, arcuate, and tuberomammillary nuclei (D'Este et al., 2001). Interestingly, some studies have suggested a role for the histaminergic system in amylin-induced anorexia (D'Este et al., 2001; Lutz et al., 1996, Mollet et al., 2001, 2003). Finally, functional amylin receptors can be derived when calcitonin receptors and receptor activity modifying protein (RAMP) are co-expressed (Christopoulos et al., 1995, 1999).

The effects of amylin on area postrema neuronal activity have been studied by using electrophysiological and immunohistochemical techniques (Riediger et al., 2001, 2004). The responses of arcuate neurons to amylin also have been studied by using extracellular recording technique in the brain slices (Davidowa et al., 2004). Although these studies demonstrated the presence of amylin responding neurons in the area postrema and the arcuate nucleus, the mechanisms of amylinergic modulation of neuronal excitability have not been established.

In the present study, we investigated the responses of area postrema neurons to amylin using patch-clamp recording methods in rat brain slices. We also assessed whether cells display I_h or not (Funahashi et al., 2002, 2003). The aim of the study was to clarify the intrinsic membrane properties of cells responsive to amylin, and to identify the mechanism(s) by which amylin modulates neuronal excitability.

2. Results

The voltage-clamp recording was performed in 66 neurons in the presence of TTX to test whether they respond to amylin or not, when amylin was applied into the bath solution or was focally applied with a micropipette using a pressure injection system. All cells were classified based on the presence or absence of electrophysiological evidence of I_h (Funahashi et al., 2002, 2003). Half of the total 66 neurons recorded displayed I_h and half did not. Excitatory responses to amylin were only observed in 11 of 33 cells not displaying I_h, i.e. no responses to amylin were seen in the cells displaying I_h (0/33 cells; $p=0.0004$, Fisher's exact test). An inhibitory effect of amylin was never seen in either cell type.

Analysis of changes in mEPSCs recorded in the presence of TTX indicated a presynaptic amylin-induced facilitation of synaptic transmission ($n=5$). A typical recording of mEPSCs is shown in Fig. 1A. This cell showed increases in the frequency of mEPSCs in response to amylin application without a marked tonic inward current. As shown in Fig. 1B, the significant increases in the frequency of mEPSCs in response to amylin (10 and 100 nM) 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 cells ($p<0.01$, KS test, $n=200$ events in each category, in both concentrations). The mean inter-event interval of mEPSCs in this neuron was significantly decreased during the application of amylin (10 and 100 nM) as compared with the value before the application of amylin (Fig. 1C right, $p<0.01$, Student's t test). The drug washout time was found to be longer in case of the higher concentration of amylin. Next, we analyzed the amplitude distribution and the mean amplitude of mEPSCs (Fig. 1D, E). Although the amplitude of mEPSCs was up to 50 pA before and during the application of 1 nM amylin, the higher concentrations of amylin (10 and 100 nM) led to mEPSCs that showed much greater amplitude (i.e. up to 190 pA). Plots of the amplitude