Electrophysiological analysis of neurons in the area postrema

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ABSTRACT : This mini-review highlights electrophysiological studies of neurons in the area postrema, including studies using anesthetized animals, neurons in the brain slices, and cultured cells. Electrophysiological studies have demonstrated chemosensitivities, intrinsic membrane properties, synaptic responses and the receptive mechanism of several peptides. For seven decades, electrophysiological research has produced much of what we know about the behavior of postrema neurons. This and other issues are presented and discussed in this paper.

Key Words : area postrema, electrophysiology, chemosensitivity, membrane property, rats

Introduction

The area postrema is located at the caudal end of the fourth ventricle in the brainstem, and it is one of the circumventricular organs that lack a blood-brain barrier. The area postrema has been implicated as an important central structure involved in the regulation of several autonomic functions including the control of food intake[1-3], body fluid homeostasis[4, 5] and cardiovascular regulation[6]. The area postrema is also well known as a trigger zone for emesis[7]. A lot of findings about the area postrema have been accumulated in the past 70 years. Now we can refer to 595 and 2163 papers that contain "area postrema" in the title and abstracts respectively. Anatomical studies have subjected the area postrema since 1946, and physiological studies including behavioral analysis have subjected the area postrema since 1957, *e.g.* Aladzhalova and Koltsova, 1964[8]. Among all possible experimental methodologies, an electrophysiological analysis is powerful to demonstrate the properties of single neurons in the area postrema, such as the neuronal excitability, intrinsic membrane properties, synaptic inputs and responsiveness to chemical substances. This review highlights studies performed by using electrophysiological techniques.

1. Extracellular recordings from neurons in the area postrema

An extracellular recording technique with metal or glass microelectrodes is useful to investigate chemosensitivity and osmosensitivity of central neurons. Initially, electrical activity of area postrema neurons was recorded by using an electroencephalogram[8-11]. The electroencephalogram recordings from the area postrema were compared with electrocorticogram[8]. Clemente et al. have investigated osmosensitivity of the area postrema neurons in 1957[9]. Some studies have investigated the effects of serotonin on the electrical activity of the area postrema neurons[10, 11]. The first report of the unit activity of area postrema neurons has appeared in the study by Borison et al. in 1975[12]. The effects of ouabain on the spike discharge rate of the area postrema neurons have been demonstrated. Around that time, chemoreceptor function of the area postrema has been vigorously investigated by using an extracellular recording technique in vivo and in vitro. Brooks *et al.* has investigated the effects of KCl, L-glutamic acid, carbamylcholine, neostigmine, serotonin, angiotensin II, and dopamine on unit discharge rate in vitro, and they found facilitatory effects of KCl and carbamylcholine[13].

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Extracellular recordings of unit discharges were also performed *in vivo* experiments to demonstrate the sensitivity of area postrema neurons to various chemical substances, such as glutamate, histamine, norepinephrine, serotonin, dopamine, apomorphine, angiotensin II, neurotensin, leucine enkephalin, vasoactive intestinal polypeptide, thyrotropin releasing hormone, gastrin, vasopressin, and substance P, with one barrel of a seven-barrel ionophoretic electrode. No responses were found to acetylcholine, somatostatin, or cholecystokinin[14]. Glucose sensing system in the area postrema was also demonstrated by using extracellular recordings of spike discharges *in vivo* and *vitro* experiments[15-17]. Co-activation by glucose and cholecystokinin (CCK-8 sulfate) was also detected in some area postrema neurons[17]. It has been demonstrated that majority of glucose-excited neurons in the area postrema also responded to amylin by using extracellular unit recordings from a rat area postrema slice preparation[18]. Ferguson *et al*. have demonstrated the responses of area postrema neurons to peptide hormones, such as endothelin, angiotensin II, vasopressin and cholecystokinin, adrenomedullin by using extracellular recording from neurons in the area postrema[6, 19-24].

2. Patch-clamp recordings from neurons in the area postrema

Since Erwin Neher and Bert Sakmann[25] have developed for measuring the extremely weak currents involved in ion transits, this method was used to analyze neurons in the area postrema. Studies performed by using the patch-clamp method demonstrated intrinsic properties of single neurons such as ion channels and synaptic currents. Analysis of single neurons in the area postrema laid basis for studying functional significance of area postrema neurons.

a. Membrane excitability

Since 1995, the patch-clamp technique has been used to investigate the membrane properties of area postrema neurons[27-31]. The paper by Hay and Lindsley is first report of membrane properties of rat area postrema neurons examined in neurons in culture[26]. The paper by Jahn *et al*., 1996[28] is first report of patch-clamp recordings from rabbit area postrema neurons in brain slices. Funahashi *et al*. firstly demonstrated intrinsic properties of membrane potentials and currents of rat area postrema neurons using the patch-clamp technique in rat brain slices. Because neuronal connectivity and synaptic activities are kept living in brain slice preparations, it enables us to investigate spontaneous and evoked synaptic currents. The presence of area postrema neurons displaying hyperpolarization-activated cation current (Ih) was firstly demonstrated by Funahashi *et al*.[31]. They also defined the functional significance of Ih for pace-making of action-potential of area postrema neurons[31].

b. Ion channels expressed in the area postrema neurons

A patch-clamp recording technique has enabled us to identify what ion channels expressed in the area postrema neurons. Voltage-dependent sodium channels and voltage-dependent potassium channels have been identified in rat area postrema neurons in 1995[26]. Hay *et al*. demonstrated tetrodotoxin (TTX)-sensitive voltage-dependent sodium currents but not TTX insensitive sodium current. They also demonstrated that the area postrema has at least two types of potassium currents, such as tetraethylamonium (TEA)-sensitive a slowly activating outward potassium current and 4-AP sensitive a rapidly inactivating I_A type current. In addition, they showed that angiotensin II attenuated both the peak and the steady-state potassium currents, and suggested that angiotensin II may modulate area postrema activity by inhibiting voltage-gated potassium currents. Depolarization-activated fast sodium current was also found in rabbit area postrema neurons[28].

Hay *et al*. have reported that a high-threshold transient current via the T-type calcium channel were found in 64% of the area postrema neurons recorded[27]. A low-threshold voltage-activated calcium current has been identified in area postrema neurons, and its channel subtype was pharmacologically identified to be neither L- nor N-types[27].

Currents via the hyperpolarization-activated cation channel (H-channel) in the area postrema neurons were firstly found in the study using a slice patch-clamp technique in rat brain slices[31, 32]. H-channel have not reported in other studies that tried to record membrane currents in cultured cells in the area postrema. Funahashi *et al*. investigated the properties of H-channel, *e.g*. activation and deactivation kinetics, and pace-making function of H-channel for spontaneous action-potential firings by using voltage- and current-clamp methods. Two distinct type of transient outward potassium currents, *i.e*.

fast and slow decay time constant and rise time, were also found in our study[30, 31].

c. Synaptic transmission

Jahn *et al*.[28] have demonstrated GABA-A receptor mediated synaptic currents of rabbit area postrema neurons. GABA receptor mediated synaptic currents have been also found in rat area postrema neurons[33]. Nicotinic Ach receptor mediated excitatory synaptic currents were found in rats[33-35]. Presence of 5-HT_3 receptors in the presynaptic terminal projecting to area postrema neurons was identified by using analysis of mEPSCs[36]. Funahashi *et al*. demonstrated that AMPA receptor mediated synaptic current was principal component of evoked EPSC[36]. ATP receptor was found to exit both pre- and post-synaptic terminals projecting to area postrema neurons[37, 38]. Modulation of neuronal excitability by ghrelin receptor has been reported[39]. Modulation of area postrema neuronal excitability by prokineticin 2, which is a brain-gut peptide related to circadian rhythm, have been suggested to have possible role for autonomic regulation[40]. Amylin receptor was elucidated to be present predominantly at the presynaptic terminal projecting to the area postrema neurons by using a amphotericin-B perforated patch-clamp technique[41]. Synaptic currents mediated by CCK receptors were analyzed by a whole-cell patch-clamp technique[42]. Responses to other peptides, *e.g*. orexin, adrenomedullin, adiponectin and ghrelin, that are elated to the regulation of food intake or body fluid homeostasis, have been demonstrated by a patch-clamp technique with brain slices or dissociated neurons.[43-46].

3. Future of the area postrema electrophysiology

An understanding of physiological role of the area postrema has grew since beginning of electrophysiological analysis. Especially, the patch-clamp technique made dramatic progress in the analysis of the membrane properties and intracellular signals of area postrema neurons. The electrophysiology is still only strategy for analysis of real-time activity of live cells with very high temporal resolution. It is the powerful tool for measuring the membrane potential and current, such as the resting potential, the action potential and current, the excitatory synaptic potential and current, and the inhibitory synaptic potential and current. Electrophysiological methods have enabled us to determine presynaptically-mediated events. A better understanding of the physiological functions of area postrema will be achieved by the electrophysiological studies combined with the behavioral experiment.

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