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Research Report

Purinergic modulation of area postrema neuronal excitability in rat brain slices

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ABSTRACT

ATP has been shown to excite neurons in various regions of the central nervous system. Whereas immunohistochemical studies show P2X receptors in the area postrema, the responsiveness of area postrema neurons to extracellular ATP has not been studied. To investigate the effects of purinoceptor activation on area postrema neuronal excitability, we performed whole-cell recordings from area postrema neurons in rat brain slices. Most area postrema neurons responded to ATP application, and most responses were excitatory. Voltage-clamp recordings showed three different types of response: (1) a postsynaptic or extrasynaptic excitatory response (inward currents; $n=26/51$ cells), (2) a presynaptic excitatory response (increased frequency of miniature excitatory postsynaptic currents with only a small direct postsynaptic current; $n=24/51$ cells, or (3) a postsynaptic inhibitory response (outward current; $n=1/51$). The excitatory responses were found in both of the two major electrophysiological cell classes, i.e. cells displaying I_h and cells not displaying I_h, while the inhibitory responses were found in only cells not displaying I_h. Current-clamp recordings showed ATP-induced depolarization ($n=13/15$) or hyperpolarization ($n=2/15$) of membrane potential that modulated the frequency of action potentials. In the presence of CNQX, mEPSCs were abolished and bath-applied ATP did not generate mEPSCs, indicating that glutamate release was facilitated by the activation of presynaptically located ATP receptors. Our pharmacological results from studies with ATP, αβme-ATP, βme-ATP and PPADS indicate that the post- and/or extrasynaptic responses are most likely mediated by $P2X_7$ receptors and/or receptors composed of $P2X_2$ and $P2X_5$ subunits. We conclude that half of the presynaptic responses are most likely mediated by $P2X_7$ receptors and/or receptors composed of $P2X_2$ and $P2X_5$ subunits while the others also contain $P2X_1$ subunits. It is well known that $P2X_7$ subunit forms only homomultimeric P2X receptors. Finally, the present study suggests that purinoceptor activation may contribute to the control of several autonomic functions by area postrema neurons.

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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. Its blood vessels lack a blood–brain barrier, offering specific central neural components unique access to circulating substances. The relation between area postrema neuronal activity and autonomic functions has been implicated by many electrophysiological and behavioral studies. Its function is regarded as being closely correlated with the autonomic regulation of food intake (Contreras et al., 1984; Van der Kooy, 1984; Ritter et al., 1986), body fluid homeostasis (Miselis et al., 1987; Iovino et al., 1988), cardiovascular regulation (Ferguson and Smith, 1991), and a chemoreceptive trigger zone for emesis (Borison and Wang, 1953). Although rats do not vomit, it is thought that the rat area postrema in this species is concerned with conditioned taste or drug-induced aversion learning associated with nausea (Berger et al., 1973; Coil and Norgren, 1981; Gallo et al., 1990).

Behavioral and electrophysiological studies have reported a relation between purinoceptors and autonomic function. Purinergic receptor activation affects the regulation of food intake (Seidel et al., 2006; Kittner et al., 2006), respiration (Antunes et al., 2005, Gourine et al., 2005a,b), circulation (Scislo and O'Leary, 2005), and body temperature (Gurin et al., 2003). Increases of ATP concentration in the ventrolateral medulla during hypoxia have been demonstrated using novel amperometric biosensors for ATP, indicating a functional significance of ATP-mediated purinergic signaling in the brainstem for the control of respiratory activity (Gourine et al., 2005a,b). Microinjection of ATP and its analogue into the NTS decreased mean arterial pressure, heart rate, and sympathetic nerve activity in rats (Scislo and O'Leary, 2005). Administration of an ATP analogue or P2X antagonists into the third ventricle caused marked changes in body temperature (Gurin et al., 2003). Although microinjection of adenosine into area postrema decreased mean arterial pressure, heart rate, and renal sympathetic nerve activity (Chen et al., 2000), effects of ATP in the area postrema have not been demonstrated.

It is well known that ATP plays an important role as a neuronal transmitter or a neuromodulator in both central and peripheral neurons. Extracellular ATP acts on specific cells via P2 purinoceptors, i.e., ionotropic P2X receptors and metabotropic P2Y receptors. Seven P2X ($P2X_{1–7}$) and eight P2Y ($P2Y_{1, 2, 4, 6, 11–14}$) subunits have been identified. Their cDNAs have been cloned, and each subunit has been pharmacologically characterized by their agonists and antagonists. P2X receptors mediate fast synaptic transmission while P2Y receptors are G-protein-coupled receptors that mediate signal transduction via the induction of inositol triphosphate (IP_3), leading to intracellular Ca^{2+} signaling. Both receptor types are present in central and peripheral neurons. Their functional roles for the control of neuronal excitability have been vigorously investigating in various regions, e.g. the spinal cord dorsal horn (Shiokawa et al., 2006), the nucleus tractus solitarius (NTS) (Kato and Shigetomi, 2001; Ueno et al., 1992a,b), hypothalamus (Matsumoto et al., 2004; Wakamori and Sorimachi, 2004), hippocampus (Pankratov et al., 1998), the dorsal root ganglion (Petruska et al., 2000), trigeminal ganglion (Khakh et al., 1997) and nodose ganglion (Nabekura et al., 1995).

In the area postrema of rats, the presence of $P2X_1$–$P2X_6$ purinoceptors has been demonstrated using the immunohistochemical method (Yao et al., 2000). $P2X_2$ receptors have been detected in vagal afferent terminals and in glutamatergic cells of the area postrema (Atkinson et al., 2000). These results suggest that ATP modulates the vagal afferent inputs to area postrema and also directly affects area postrema neuronal excitability. Electrophysiological studies of ATP and purinoceptor activation in the area postrema, however, are virtually absent.

In the present study, we investigated the purinergic modulation of area postrema neuronal excitability using patch-clamp techniques in rat brain slices. The principal aim of the present study was to define the mechanism of ATP-induced modulation of neuronal excitability in the area postrema by: (1) determining the responsiveness of rat area postrema neurons to purinergic receptor activation; (2) determining the electrophysiological cell classes that exhibit responsiveness to purinergic receptor activation; and (3) estimating which receptor subunits comprise purinoceptors in area postrema neurons.

2. Results

2.1. *ATP-induced responses and electrophysiological cell types*

Most area postrema neurons responded to ATP application, and most responses were excitatory. Responses to bath application of ATP were found in approximately 62% of neurons tested (n=66/106). In responding cells, the vast majority (63/66) were excited by ATP, and inhibitory responses were found in 3 neurons. Forty cells showed no response to ATP application.

There are two major subclasses that characterized by the presence or absence of a hyperpolarization-activated cation current (I_h; Funahashi et al., 2006). We classified all recorded neurons according to their intrinsic membrane properties to be able to correlate ATP-responsiveness with their electrophysiological subclass. Excitatory responses to ATP were found in both neuron classes (i.e. displaying I_h or not displaying I_h), whereas inhibitory responses to ATP were found only in neurons that did not display I_h (Table 1).

Typical responses to the bath application of ATP recorded in voltage-clamp mode at a holding potential of −50 mV are shown in Fig. 1. Bath-applied ATP elicited one of two types of excitatory responses. Either there was a marked postsynaptically induced inward current (e.g. 160 pA) (n=26/51,

Table 1 – Effects of purinoceptor activation in area postrema neurons

Effect	n	I_h (+)	I_h (–)
Excitatory	63	21	42
Inhibitory	3	0	3
No change	40	26	14

I_h: hyperpolarization-activated cation current; I_h (+): cells displaying I_h; I_h (–): cells not displaying I_h.