Acute stress diminishes M-current contributing to elevated activity of hypothalamic-pituitary-adrenal axis

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A B S T R A C T

Acute stress stimulates corticotrophin-releasing hormone (CRH)-expressing neurons in the hypothalamic paraventricular nucleus (PVN), which is an essential component of hypothalamic-pituitary-adrenal (HPA) axis. However, the cellular and molecular mechanisms remain unclear. The M-channel is a voltage-dependent K+ channel involved in stabilizing the neuronal membrane potential and regulating neuronal excitability. In this study, we tested our hypothesis that acute stress suppresses expression of Kv7 channels to stimulate PVN-CRH neurons and the HPA axis. Rat PVN-CRH neurons were identified by expressing enhanced green fluorescent protein driven by Crh promoter. Acute restraint stress attenuated the excitatory effect of Kv7 blocker XE-991 on the firing activity of PVN-CRH neurons and blunted the increase in plasma corticosterone (CORT) levels induced by microinjection of XE-991 into the PVN. Furthermore, acute stress significantly decreased the M-currents in PVN-CRH neurons and reduced PVN expression of Kv7.3 subunit in the membrane. In addition, acute stress significantly increased phosphorylated AMP-activated protein kinase (AMPK) levels in the PVN tissue. Intracerebroventricular injection of the AMPK inhibitor dorsomorphin restored acute stress-induced elevation of CORT levels and reduction of membrane Kv7.3 protein level in the PVN. Dorsomorphin treatment increased the M-currents and reduced the firing activity of PVN-CRH neurons in acutely stressed rats. Collectively, these data suggest that acute stress diminishes Kv7 channels to stimulate PVN-CRH neurons and the HPA axis potentially via increased AMPK activity.

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1. Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is critical in maintaining homeostasis as the body responds to environmental stressor (Lupien et al., 2009; McEwen, 2007; Pedersen et al., 2001).

The HPA axis include the paraventricular nucleus (PVN) of the hypothalamus, which secretes corticotrophin-releasing hormone (CRH) and arginine vasopressin; the pituitary gland which releases corticotrophin (ACTH), a process triggered by CRH and arginine vasopressin; and the adrenal gland cortex, that secretes the glucocorticoids (Goncharova, 2013). As a key component of the HPA axis in basal conditions and in response to stress, the PVN-CRH neurons synthesize and release CRH, a peptide of 41 amino acid residues, and project to the median eminence (Vale et al., 1981), where CRH is released into the portal system of the pituitary (Aguilera and Liu, 2012). Acute restraint stress causes an increase in CRH mRNA levels and c-fos expression in the CRH neurons in the PVN (Day et al., 2005; Girotti et al., 2006; Imaki et al., 1998). However, the cellular mechanisms underlying the hyperactivity of the PVN-CRH neurons under stress conditions are not clear.

It has been challenging to functionally analyze CRH neuronal...
activity until recently, when a genetic approach to tagging CRH neuron by expressing green fluorescent protein (GFP) in transgenic mouse line was developed (Alon et al., 2009; Itoi et al., 2014; Martin et al., 2010; Vanmeele Cusulin et al., 2013). To target CRH neurons in rat PVN, we used a recently developed approach for reliably express enhanced GFP (eGFP) driven by rat Crh promoter (Gao et al., 2017). The intrinsic neuronal excitability is tightly controlled by the transmembrane ionic currents including M-current, a voltage-gated and non-inactivating K⁺ current (Brown and Adams, 1980; Delmas and Brown, 2005; Marrion, 1997; Peters et al., 2005). The M-current stabilizes the membrane potential and helps maintain the resting membrane potential of neurons (Brown and Adams, 1980). Kcnq genes encode Kv7.1–7.5 K⁺ channel subunits, which form Kv7 channels (Brown and Yu, 2000; Brown and Adams, 1980). Genetic ablation of or acute inhibition of Kv7 channels leads to depolarization and excitation, whereas opening of Kv7-channels results in hyperpolarization and inhibition of neurons. The neuronal M-current is predominantly carried by heterotetrameric Kv7.2 and Kv7.3 subunits (Shah et al., 2002; Wang et al., 1998). Dysfunction of Kv7-channels results in several neuron-generated diseases including epilepsy, pain, memory deficit/decline, and depression (Cavaliere et al., 2013; Passmore et al., 2003; Qi et al., 2014; Zhang et al., 2013). The Kv7-channel is also involved in the regulation of a stress-related neuronal process. In this regard, activation of Kcnq/Kv7 channels prevents acute stress-induced impairments of hippocampal long-term potentiation and spatial memory retrieval in rats (Li et al., 2014).

AMP-activated protein kinase (AMPK) is a ubiquitous serine/threonine kinase which is involved in cellular responses to many metabolic stresses (Kim et al., 2009). AMPK is involved in many cellular processes, such as regulation of apoptosis, stimulation of autophagy and phagocytosis, inhibition of cell growth and proliferation, and counteraction of hypertrophy (Dermaku-Sopjani et al., 2014; Hardie, 2003). Acute restraint stress increases AMPK activity (Marques et al., 2012) and AMPK activation in the central nervous system mediates fructose-induced elevation of plasma corticosterone (CORT) levels (Kinote et al., 2012). Furthermore, AMPK activation decreases membrane expression of Kv7.1 and epithelial Na⁺-channel through promoting endocytosis and degradation in lysosomes via a Nedd2-4-dependent mechanism (Andersen et al., 2012; Bhalla et al., 2006). Nedd4-2 is an ubiquitin ligase that ubiquitylates membrane proteins to increase protein internalization and degradation (Abriel and Staub, 2005). Nedd4-2 suppresses Kv7.2/7.3-mediated M-currents (Ekberg et al., 2007), indicating that AMPK-Nedd4-2 is a potential pathway through which acute stress regulates Kv7 channels in PVN-CRH neuron. Thus, in this study, we tested the hypothesis that acute stress suppresses the Kv7 channels to stimulate CRH neurons through activation of AMPK.

2. Method and materials

Male Sprague-Dawley rats (12-week old) were used in this study. The rats were group-housed (n = 3 rats per cage) in a 12-h light/dark cycle and maintained under controlled temperature (24–25 °C) with food and water ad libitum. The surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals.

2.1. Acute restraint stress in rats

Restraint stress was designed to restrict the rat's movement without promoting any apparent signs of pain. To avoid diurnal fluctuations of CORT, the restraint procedure was performed at 10:00 a.m. in all rats. The acute restraint stress started with a 30-min equilibration period that allowed the rats adjust to their new surroundings. Upon completion of this period, each rat was placed into a 5-cm diameter Plexiglas tube and their movement was limited in its ability to turn around by an adjustable plastic plug that was secured behind the rat in the tube for a period of 2 h. Fresh air was accessible to the Plexiglas tube through holes on the sides of the tube. Food and water were not provided to the rats during the restraint procedure (Sweerts et al., 1999). Blood samples were collected from a saphenous vein before restraint and at multiple time points (0, 20, 40, 60, and 90 min) after restraint. To avoid the influence of blood volume loss on hormone regulation, small amounts (100 μl) of blood were collected. CORT levels were measured by using an ELISA kit from Enzo Life Science (Farmingdale, NY) according to the manufacturer's instructions.

2.2. PVN injections

Under anesthesia with isoflurane (2%), the head of the rats were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). A bilateral guide cannula (26-gauge, 1.0-mm spacing between cannula, and extending 6.8 mm from the pedestal) was implanted at the following coordinates: 1.8 mm caudal to the bregma, 0.5 mm lateral to the midline, and 6.8 mm ventral to the surface of the dura (1.0-mm dorsal to the intended drug injection site). The bilateral guide cannula for PVN injection was affixed to the skull by using dental acrylic, a dummy cannula was inserted into each side of the guide cannula, and a dust cap was then placed over the external end of the dummy cannula. After a 1-week recovery period, the dummy cannula was removed and a bilateral injection cannula with tips protruding 1.0 mm beyond the tip of the guide cannula was inserted into the guide cannula (Gao et al., 2017). Kv7 blocker 10, 10-bis (4-pyridinylmethyl)-9(10H)-anthracnose (XE-991, 0.5 pmol in 100 nl of aCSF) was dissolved in aCSF and injected bilaterally into the PVN. To mark the infusion sites, fluorescent microspheres (0.04 μm, wavelength 580 nm) were delivered through the cannula after XE-991 injection.

2.3. PVN-CRH neuron identification

We selectively identified CRH-expressing neurons in rat PVN by expressing enhanced green fluorescent protein (eGFP) driven by rat Crh promoter (Gao et al., 2017). The full-length promoter fragment (–2125/+94) and necessary components were subcloned into the adeno-associated virus (AAV, serotype 1/2) vector expression cassette. The packaging was performed by Gene detect, Ltd (Auckland, New Zealand) with a titer of 10^12 ^fl^76 /μl. The surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals. The rats were injected intracerebroventricularly with 50–60 mg of colchicine (Gao et al., 2017; Sawchenko et al., 1984). Briefly, rats were deeply anesthetized (with sodium pentobarbital 50 mg/kg by intraperitoneal injection) and rapidly perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). The