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AMP KINASE REGULATES LIGAND-GATED K-ATP CHANNELS IN SUBSTANTIA NIGRA DOPAMINE NEURONS

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Abstract—AMP-activated protein kinase (AMPK) is a master enzyme that regulates ATP-sensitive K^+ (K-ATP) channels in pancreatic beta-cells and cardiac myocytes. We used patch pipettes to record currents and potentials to investigate effects of AMPK on K-ATP currents in substantia nigra compacta (SNC) dopamine neurons in slices of rat midbrain. When slices were superfused repeatedly with the K-ATP channel opener diazoxide, we were surprised to find that diazoxide currents gradually increased in magnitude, reaching 300% of the control value 60 min after starting whole-cell recording. However, diazoxide current increased significantly more, to 472% of control, when recorded in the presence of the AMPK activator A769662. Moreover, superfusing the slice with the AMPK blocking agent dorsomorphin significantly reduced diazoxide current to 38% of control. Control experiments showed that outward currents evoked by the K-ATP channel opener NN-414 also increased over time, but not currents evoked by the $GABA_B$ agonist baclofen. Delaying the application of diazoxide after starting whole-cell recording correlated with augmentation of current. Loose-patch recording showed that diazoxide produced a 34% slowing of spontaneous firing rate that did not intensify with repeated applications of diazoxide. However, superfusion with A769662 significantly augmented the inhibitory effect of diazoxide on firing rate. We conclude that K-ATP channel function is augmented by AMPK, which is activated during the process of making whole-cell recordings. Our results suggest that AMPK and K-ATP interactions may play an important role in regulating dopamine neuronal excitability. Published by Elsevier Ltd on behalf of IBRO.

Key words: AMP kinase, diazoxide, substantia nigra, brain slice, K-ATP, patch-clamp.

INTRODUCTION

AMP-activated protein kinase (AMPK) is a complex enzyme composed of an alpha catalytic subunit and beta and gamma regulatory subunits (Hardie et al., 2003). Widely expressed in brain and somatic tissues, AMPK is activated by rising levels of AMP that displaces ATP at the gamma subunit, which produces a conformational change that enables phosphorylation of the alpha subunit at Thr-172 (Oakhill et al., 2010). Once AMPK is bound by AMP, the upstream kinases liver kinase B1 (LKB1) and calcium-calmodulin-dependent kinase kinase beta (CaM-KKβ) phosphorylate AMPK and cause AMPK activity to increase many fold (Amato and Man, 2011). In peripheral tissues such as liver and skeletal muscle, AMPK encourages processes that increase ATP production, such as increasing glucose uptake, accelerating glycolysis, and increasing the oxidation of fatty acids. Furthermore, AMPK inhibits pathways that consume ATP, such as the syntheses of fatty acids, glycogen and proteins. (Russo et al., 2013). Conditions that deplete energy stores, such as hypoglycemia, hypoxia, ischemia and calorie restriction, also activate AMPK (Hardie et al., 2003; Chiba et al., 2010). Thus, AMPK plays an important role in the conservation of cellular energy and in replenishing the supply of high-energy compounds.

AMPK is often linked to ATP-sensitive K^+ (K-ATP) channels because both respond to alterations in metabolic conditions (Nichols, 2006; Yoshida et al., 2012). The K-ATP channel is a hetero-octameric complex composed of a sulfonylurea binding protein (SUR) and an inwardly rectifying K^+ channel (Kir) (Nichols, 2006). K-ATP channels are blocked by rising ATP levels and are opened with falling energy levels. In pancreatic beta-cells, AMPK increases the expression of K-ATP channels on the cell surface and thereby facilitates insulin secretion (Smith et al., 2006; Lim et al., 2009). AMPK has also been shown to promote K-ATP channel opening in pancreas and cardiac myocytes (Yoshida et al., 2012; Beall et al., 2013). K-ATP channels in pancreas are composed of SUR1 and Kir6.2 subunits, which are also widely expressed in the brain (Aguilar-Bryan and Bryan, 1999). Although AMPK and K-ATP channels are widely expressed in the brain, little is known of their interactions. Recently, our lab reported that K-ATP current could be generated by N-methyl-D-aspartate (NMDA) receptor stimulation through a Ca^{2+}-dependent activation of AMPK in subthalamic nucleus (STN) neurons (Shen et al., 2014). This finding is important because the inhibitory influence of K-ATP current may tend to

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Abbreviations: AMPK, AMP-activated protein kinase; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; K-ATP channel, ATP-sensitive K^+ channel; NMDA, N-methyl-D-aspartate; SNC, substantia nigra zona compacta; STN, subthalamic nucleus; SUR, sulfonylurea binding protein.

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counterbalance the excitatory influence of glutamate receptor stimulation. Moreover, others have shown that K-ATP currents can modulate the firing pattern in a subset of dopamine neurons in rodent midbrain (Schiemann et al., 2012). These results suggest that K-ATP channels and their regulation by AMPK may be important for altering neuronal excitability and firing pattern.

Dopamine neurons in the substantia nigra zona compacta (SNC) play vital roles in goal-directed behaviors and behavioral reinforcement as well as in enabling normal movement. Because firing pattern is important in modifying dopamine release (Grace et al., 2007; Paladini and Roeper, 2014), a better understanding of how ion channels and membrane properties modify neuronal excitability could lead to better control of dopamine-dependent behaviors. The purpose of the present study was to characterize AMPK and K-ATP channel interactions in SNC dopamine neurons. Using patch-pipette recordings in slices of rat midbrain, we found that AMPK activation during whole-cell recordings cause gradual augmentation of ligand-gated K-ATP currents. Moreover, inhibition of AMPK activity caused rundown of K-ATP currents, suggesting that AMPK activity is necessary for normal K-ATP function. Our results suggest that an AMPK and K-ATP interaction may play an important role in regulating dopamine neuronal excitability.

EXPERIMENTAL PROCEDURES

All procedures were approved by the Institutional Animal Care and Use Committee at the Veterans Affairs Portland Health Care System. Every precaution was taken to minimize animal stress and the number of animals used. Tissue preparation and whole-cell recordings were done as reported previously by our lab (Shen et al., 2014).

Tissue preparation

A total of 96 male Sprague–Dawley rats (80–180 g, Harlan, Indianapolis, IN, USA) were used in our studies. Briefly, rats were anesthetized with isoflurane and euthanized by severing major thoracic vessels. Brains were rapidly removed and horizontal slices were cut with a vibratome (200–300 μm thick) in an ice-cold sucrose buffer solution of the following composition (in mM): sucrose, 196; KCl, 2.5; $MgCl_2$, 3.5; $CaCl_2$, 0.5; NaH_2PO_4, 1.2; glucose, 20; and $NaHCO_3$, 26, equilibrated with 95% O_2 and 5% CO_2. A slice containing the SNC was then placed on a supporting net and submerged in a continuously flowing solution (2 ml/min) of the following composition (in mM): NaCl, 126; KCl, 2.5; $CaCl_2$, 2.4; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 19; glucose, 11, gassed with 95% O_2 and 5% CO_2 (pH 7.4) at 36 °C. A low-power microscope was used to identify the SNC as gray matter rostral and caudal to the medial terminal nucleus of the accessory optic tract.

Electrophysiological recordings

Whole-cell recordings were made with pipettes containing (in mM): potassium gluconate, 138; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; ATP, 1.5; GTP, 0.3 (pH 7.3). Pipette resistance ranged from 3 to 8 MΩ, whereas series resistance typically ranged from 15 to 40 MΩ. In some experiments, we used a "loose-patch" method to record extracellular potentials (Nunemaker et al., 2003). For loose-patch recordings, patch pipettes were filled with standard extracellular solution; patch resistance was typically 5–10 times the starting value. Most recordings were performed with the "blind" patch method in which slices were visualized with a dissection microscope (Leica StereoZoom-7, IL, USA) and whole-cell tight-seal recordings were made without visualization of individual neurons and without any cleaning of the slice surface (Blanton et al., 1989). Visualized-patch recordings utilized an upright Zeiss Axioskop (CA, USA) microscope equipped with infra-red Nomarski optics to allow for visualization of individual neurons (Stuart et al., 1993). Membrane currents or potentials were amplified and recorded with an Axopatch-1D amplifier with 5-kHz low-pass filter (Molecular Devices, Foster City, CA, USA). Data were acquired using a personal computer with a Digidata analog/digital interface and analyzed using pCLAMP 10 software (Molecular Devices/Axon Instruments, Foster City, CA, USA). Dopamine neurons were identified by their broad action potentials or currents and by inhibitory effects of dopamine (30 μM) on membrane potential or current. Membrane potentials for whole-cell recordings were corrected for the liquid junction potential (10 mV).

Current–voltage studies

Current–voltage (*I–V*) relationships were constructed by measuring currents during a series of hyperpolarizing voltage steps (400 ms duration) with 10-mV increments from a holding potential of −70 mV. Currents were measured immediately after capacitive transients to minimize the influence of hyperpolarization-activated cation currents. When measuring the voltage dependence of diazoxide currents, *I–V* plots were always obtained at the end of the 5-min application of diazoxide. *I–V* plots are displayed as "subtracted" currents in which currents recorded during the experimental treatment were subtracted from those currents recorded in the control condition. Therefore, these subtracted currents represent "net" currents that were produced or blocked by an experimental treatment. Slope conductance was calculated for each cell as the slope of a straight line in the *I–V* plot at voltages between −80 and −120 mV. Mean slope conductance and S.E.M. were obtained by averaging slope conductance from all cells in each experimental group.

Drugs and chemicals

All drugs were dissolved in aqueous or dimethyl sulfoxide stock solutions. Most drugs were added to the slice superfusate. Stock solutions were diluted at least