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Differential actions of AMP kinase on K-ATP currents in ventral tegmental area and substantia nigra zona compacta neurons

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Abstract

ATP-sensitive K⁺ (K-ATP) channels play significant roles in regulating the excitability of dopamine neurons in the substantia nigra zona compacta (SNc). We showed previously that K-ATP channel function is up-regulated by AMP-activated protein kinase (AMPK). The current study extended these studies to the neurons adjacent to the SNc in the ventral tegmental area (VTA). Using patch pipettes to record whole-cell currents in slices of rat midbrain, we found that the AMPK activator A769662 increased the amplitude of currents evoked by the K-ATP channel opener diazoxide in presumed dopamine-containing VTA neurons. However, current evoked by diazoxide with A769662 was significantly smaller in VTA neurons compared to SNc neurons. Moreover, a significantly lower proportion of VTA neurons responded to diazoxide with outward current. However, A769662 was able to increase the incidence of diazoxide-responsive neurons in the VTA. In contrast, A769662 did not potentiate diazoxide-evoked currents in presumed non-dopamine VTA neurons. These results show that AMPK activation augments K-ATP currents in presumed dopamine neurons in the VTA and SNc, although diazoxide-evoked currents remain less robust in the VTA. We conclude that K-ATP channels may play important physiological roles in VTA and SNc dopamine neurons.

Graphical Abstract

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Keywords

ATP-sensitive potassium channel; dopamine neuron; diazoxide; AMP kinase; patch clamp recording

Introduction

Dopamine-containing neurons in the ventral midbrain play pivotal roles in an array of important behaviors. Neurons in the substantia nigra zona compacta (SNc) primarily innervate the striatum and enable movement as well as facilitate habit formation and promote stereotypy (DeLong & Georgopoulos, 1981; Lee *et al.*, 1995; Yin & Knowlton, 2006). Ventral tegmental area (VTA) dopamine neurons innervate mesolimbic structures and are linked to behavioral motivation, mood and propensity to engage in drug abuse (Le Moal & Simon, 1991; Brown *et al.*, 2012). Both regions have been implicated in mediating behavioral reward and learning (Ljungberg *et al.*, 1992; Schultz, 1998). Understanding how dopamine neurons are regulated by biological mechanisms is important for understanding how dopamine-dependent behaviors might be modified.

Activity in SNc and VTA neurons is regulated by synaptic inputs that contain glutamate, GABA and other neurotransmitters. However, intrinsic ion channels also exert significant influences on neuronal activity. Our laboratory has recently investigated the influence of ATP-sensitive K⁺ (K-ATP) channels on dopamine neuronal excitability. The K-ATP channel is a heteromeric complex composed of a sulfonylurea receptor (SUR), which serves as a regulatory subunit, and an inwardly rectifying K⁺ (Kir) channel (Aguilar-Bryan & Bryan, 1999). Most central neurons express SUR1 and Kir6.2 subunits, as is the case for ventral midbrain neurons (Karschin *et al.*, 1997; Dunn-Meynell *et al.*, 1998). K-ATP channels are regulated by many intracellular second messenger systems, but we have been interested lately in regulation of K-ATP by AMP-activated protein kinase (AMPK). AMPK has been shown to up-regulate the function of K-ATP channels in a variety of somatic tissues including pancreas and cardiac myocytes (Lang & Föller, 2014). Although less is known about K-ATP channel physiology in midbrain dopamine neurons, studies have shown that K-ATP channels can play a significant role in regulating excitability and firing pattern of SNc neurons (Schiemann *et al.*, 2012; Shen *et al.*, 2016). Moreover, prolonged activation of K-ATP channels can increase the risk of death in SNc neurons (Liss *et al.*, 2005; Toulorge *et al.*, 2010). Thus, there is much in the literature to suggest that K-ATP channels in SNc neurons might play significant roles in health and disease (Duda *et al.*, 2016).

Our laboratory recently published a study showing that outward current evoked by K-ATP channels is potentiated by AMPK activation in SNc dopamine neurons (Shen *et al.*, 2016). Curiously, our study also showed that currents evoked by the K-ATP channel opener diazoxide gradually increased in amplitude over the duration of patch pipette recording in whole cell configuration. The fact that gradual potentiation of K-ATP currents was blocked by the AMPK blocking agent dorsomorphin (Compound C) suggested that K-ATP current amplification during whole-cell recording was mediated by activation of AMPK. Although the mechanism for K-ATP current augmentation has not been fully defined as of yet, K-ATP

channel regulation in the SNC clearly differs from that seen in other central neurons. For example, previous studies in our lab showed that diazoxide-induced currents do not augment over time during whole-cell recordings of rat subthalamic nucleus (STN) neurons (Shen *et al.*, 2014). Furthermore, AMPK activation did not increase diazoxide-induced currents in STN neurons. Instead, we found that AMPK activation increased calcium-dependent K-ATP current that was evoked by glutamate receptor stimulation of STN neurons (Shen *et al.*, 2014). Moreover, calcium-dependent K-ATP current evoked by glutamate receptor stimulation in STN neurons was not observed in SNC dopamine neurons or in non-dopamine neurons in the substantia nigra zona reticulata (SNR) (Shen & Johnson, 2010; Shen & Johnson, 2013). These results suggest that K-ATP channel regulation differs according to neuronal type.

The present study was undertaken to characterize K-ATP currents and their regulation by AMPK in VTA neurons and compare results to those in SNC dopamine neurons. Our studies also included investigations of K-ATP currents in presumed non-dopamine neurons in the VTA. Although we found that most presumed dopamine neurons in the VTA showed augmentation of K-ATP currents by AMPK activation, their responses were less robust than those found in SNC neurons. Nevertheless, our data suggests that K-ATP channels may play important physiological roles in VTA dopamine neurons.

Materials and methods

Animals and tissue preparation

A total of 112 adult male rats were used in this study. Animal care and procedures were performed according to a protocol approved and monitored by the Institutional Animal Care and Use Committee at the Veterans Affairs Portland Health Care System. Care was taken to minimize animal stress and the number of animals used. Male Sprague-Dawley rats (100–180 g) were obtained from Harlan (Indianapolis, IN, USA). Rats were anesthetized with isoflurane and euthanized by severing major thoracic vessels. Brains were removed rapidly, placed in ice-cold, oxygenated sucrose buffered solution and a block of tissue containing the midbrain was placed in a vibratome (Technical Products International, St. Louis, MO, USA). The sucrose buffered solution contained (in mM): sucrose (196); KCl (2.5); MgCl₂ (3.4); CaCl₂ (0.5); NaH₂PO₄ (1.2); glucose (20); and NaHCO₃ (26), equilibrated with 95% O₂ and 5% CO₂. A 300 µm thick horizontal slice containing the ventral midbrain was placed on a supporting net and submerged in a continuously flowing (2 ml/min) heated (34–35°C) solution of the following composition (in mM): NaCl (126); KCl (2.5); CaCl₂ (2.4); MgCl₂ (1.2); NaH₂PO₄ (1.2); NaHCO₃ (19); glucose (11), gassed with 95% O₂ and 5% CO₂ (pH 7.4). Using a dissection microscope, the SNC was located as gray matter rostral and caudal to the medial terminal nucleus of the accessory optic tract; recordings were made in the lateral VTA, which was located medial to the medial terminal nucleus (Paxinos & Watson, 1986).

Whole-cell recordings

Glass micropipettes were used for whole-cell patch-clamp recordings. Internal solution (pH 7.3) contained (in mM): potassium gluconate (138); MgCl₂ (2); CaCl₂ (1); EGTA (11);

HEPES (10); ATP (1.5), GTP (0.3). Pipette resistance ranged from 3–5 M Ω , and data were discarded if series resistance was greater than 30 M Ω . Membrane currents were recorded with an Axopatch-1D amplifier and Digidata 1550 analog/digital converter that were coupled to a personal computer running pClamp 10 software (Molecular Devices, Foster City, CA, USA). Resting cellular voltage was clamped at –60 mV, which required passing nearly zero current. H-current was evoked by recording current during a 400 msec hyperpolarizing voltage step to –120 mV. H-current and action potentials were low-pass filtered at 2 kHz and sampled at 10 kHz. Long-term current recordings were sampled at 500 Hz and acquired using Axoscope software (Molecular Devices). Net diazoxide-induced current was measured as the difference between peak current and the current immediately before superfusion with diazoxide. Membrane currents were corrected for the liquid junction potential (10 mV).

Cell identification

Neurons in the VTA and SNC were classified as “principal” and “secondary” cells based upon electrophysiological and pharmacological criteria as described by Johnson and North (1992). Presumed dopamine-containing neurons (principal cells) were identified by well-established criteria (Grace & Bunney, 1983), such as the occurrence of broad, spontaneous action potentials, presence of significant H-current, and outward current in response to superfused dopamine (30 μ M). Presumed non-dopamine neurons in the VTA (secondary cells) were identified by their relatively narrow spike widths, lack of H-current, and lack of significant outward current in response to dopamine (Johnson & North, 1992). Although many of these secondary cells have been shown to be non-dopaminergic, studies have shown that some dopamine-containing neurons in the VTA may have electrophysiological characteristics typical of non-dopaminergic cells (Ungless & Grace, 2012). Also, a minority of non-dopamine neurons in the VTA may have electrophysiological characteristics of dopamine-containing neurons (Roeper, 2013). Thus, some degree of heterogeneity exists within categories of principal and secondary neurons. All neurons recorded in the SNC satisfied the criteria for presumed dopamine-containing (principal) cells.

Drugs and chemicals

Drugs and chemicals were first dissolved in aqueous or dimethyl sulfoxide stock solutions before being added to the slice superfusate. Stock solutions were diluted at least 1:1000 in the superfusate immediately prior to use. Our previous experience showed that this dilute concentration of dimethyl sulfoxide had no effect on membrane conductance. Drugs delivered via superfusion passed through a heat exchanger and entered the slice chamber within 30 sec. In some experiments A769662 was added directly to the internal pipette solution and was delivered to the intracellular contents by passive dialysis. Dorsomorphin (Compound C) and 6,7-dihydro-4-hydroxy-3-(2'-hydroxy[1,1'-biphenyl]-4-yl)-6-oxo-thieno[2,3-b]pyridine-5-carbonitrile (A769662) were obtained from Tocris Cookson Inc. (Bristol, UK), whereas dopamine HCl was obtained from Sigma-Aldrich (St. Louis, MO, USA). Diazoxide was purchased from Cayman Chemical (Ann Arbor, MI, USA).

Data analysis

Numerical data are expressed as means and standard errors of the mean. Statistical analyses were done using IBM SPSS Statistics version 22 (IBM North America, New York, NY, USA). Parametric data were tested using paired or unpaired Student's *t* tests, whereas non-parametric data were tested using Chi-Square or Fisher's Exact tests. Data tested using ANOVA were logarithmically transformed in order to pass normality tests. Therefore, data evaluated by ANOVA are displayed graphically on semi-log plots. Significance was accepted with $P < 0.05$.

Results

Diazoxide evokes K-ATP currents in VTA and SNC principal cells

Midbrain slices were superfused with the K-ATP channel opener diazoxide (200 μ M) for 5 min every 20–30 min while recording currents under voltage clamp in whole-cell configuration. As we reported previously (Shen *et al.*, 2016), the K-ATP channel opener diazoxide evoked outward currents in all principal (presumed dopamine-containing) neurons in the SNC. Moreover, repeated applications of diazoxide evoked increasingly larger outward currents in the vast majority of SNC neurons, as illustrated in Fig. 1A. Similar responses to diazoxide were recorded in VTA principal neurons, as shown in Fig. 1B. This figure also shows that the K-ATP channel blocker tolbutamide (100 μ M) causes a nearly complete block of diazoxide-evoked currents. K-ATP currents in VTA principal cells were generally smaller than those in the SNC, as has been reported previously (Liss *et al.*, 2005).

We quantified effects of tolbutamide on diazoxide-induced currents in SNC and VTA principal neurons by superfusing slices with diazoxide every 20 min and superfusing tolbutamide during the third diazoxide application. As shown in Fig. 1C, tolbutamide caused nearly complete block of diazoxide-induced current. On average, the second application of diazoxide evoked 152 ± 33 pA of outward current in SNC principal cells, but the third application of diazoxide evoked virtually no outward current (1 ± 2 pA) in the presence of tolbutamide ($n = 6$). Similarly, the second application of diazoxide evoked 60 ± 15 pA of outward current in VTA principal neurons, whereas the third application of diazoxide evoked only 3 ± 2 pA in tolbutamide ($n = 5$). These results confirm that diazoxide evokes sulfonyleurea-sensitive K-ATP currents in SNC and VTA principal cells.

Although diazoxide-evoked currents increased with repeated applications, repeated applications of tolbutamide failed to evoke substantial inward currents over time in the absence of diazoxide. In six SNC principal cells, an initial 5-min superfusion of tolbutamide (100 μ M) evoked 4 ± 4 pA of inward current, which was not demonstrably different from the 8 ± 5 pA of inward current evoked by tolbutamide 60 min after starting whole-cell recording. This result agrees with our previous finding that recording in the whole-cell configuration increases the capacity for evoking K-ATP current over time, but K-ATP current does not appear spontaneously without the application of a K-ATP channel opener (Shen *et al.*, 2016).

Variability in responses to diazoxide

Although Fig. 1 shows typical responses to diazoxide in SNC and VTA principal cells, responses were more variable in the VTA compared to the SNC. In order to quantify the responsivity to diazoxide in the VTA and SNC, responses to diazoxide were divided into three categories: 1) “no response” was defined as having less than 10 pA of outward current in response to diazoxide, 2) “no potentiation” was defined as having outward current of at least 10 pA but current increased by less than 50% within one hour, or 3) “potentiation” as defined by diazoxide-evoked current that increased by at least 50% during the first 60 min after beginning recording. As seen in Fig. 2A, all SNC neurons responded to diazoxide, although 11% (3 of 28) failed to show an increase in current over 1 hour of recording. In contrast, Fig. 2B shows that 25% of VTA dopamine neurons (9 of 36) had no response to diazoxide, and another 25% showed significant diazoxide-evoked current but no potentiation of current over 1 hour. There was a statistically significant difference in diazoxide responses in VTA compared to SNC ($P = 0.002$, $X^2 = 12.33$, Pearson Chi-Square test). Moreover, there were significantly more neurons that did not respond to diazoxide in the VTA compared to the SNC ($P = 0.004$, Fisher’s Exact test).

Diazoxide-evoked currents are potentiated by AMPK activator A769662

In some experiments, principal neurons were recorded with pipettes that contained the AMPK activator A769662 (5 μ M). In the SNC, 100% of neurons (17 of 17) showed potentiation of diazoxide-evoked current by 50% or more when recording with A769662 in pipettes (Fig. 2C). In VTA principal neurons, A769662 reduced the incidence of neurons unresponsive to diazoxide from a control incidence of 25% (9 of 36) to an incidence of 4% (1 of 27 cells; $P = 0.034$, Fisher’s Exact test; Fig. 2D). These results suggest that AMPK activation increases the responsiveness of VTA principal neurons to a K-ATP channel opener.

Inhibition of diazoxide-evoked currents by dorsomorphin

We reported previously that potentiation of diazoxide-evoked currents during whole-cell recording is reversed by the AMPK inhibitor dorsomorphin (Compound C) in SNC principal neurons (Shen *et al.*, 2016). We proceeded to test whether or not potentiation of diazoxide-evoked current was also sensitive to dorsomorphin in VTA principal neurons. Diazoxide (200 μ M) was superfused for 5 min every 30 min for three applications. Dorsomorphin (10–30 μ M) was added to the superfusate 10 min before the third diazoxide application. As shown in Fig. 3, the amplitude of the second diazoxide-evoked current increased to an average of $343 \pm 96\%$ of the initial diazoxide-evoked current. However, current evoked by the third application of diazoxide was reduced to $120 \pm 30\%$ of the initial current in the presence of dorsomorphin ($P = 0.043$, paired t test). This result suggests that potentiation of diazoxide-evoked current during whole-cell recording is dependent upon AMPK activation, as we showed previously in SNC neurons.

Diazoxide-evoked currents over time in SNC and VTA

Further comparisons between principal neurons in the SNC and VTA were done after excluding principal neurons in the VTA that did not respond to diazoxide. Initial diazoxide-

evoked current in SNC neurons (49 ± 6 pA, $n = 28$) was not significantly different from that in VTA neurons (65 ± 11 pA, $n = 27$; $P = 0.185$, t test). However, diazoxide-evoked current in SNC neurons (139 ± 20 pA, $n = 17$) was significantly greater than that in VTA neurons (88 ± 15 pA, $n = 18$) when measured 90 min after beginning recording ($P = 0.046$, t test).

We subsequently analyzed diazoxide-evoked currents in the presence and absence of A769662 after expressing currents as percentages of initial diazoxide-evoked current for each principal neuron recorded in the SNC and VTA. Figure 4A shows that diazoxide-evoked currents increased significantly more over time in SNC neurons compared to currents recorded in VTA neurons ($P = 7.2 \times 10^{-10}$, $F_{(1,137)} = 43.93$, two-way ANOVA, followed by Sidak multiple comparison tests). Figure 4B, which illustrates data recorded with A769662 in pipettes, shows that augmentation of diazoxide-evoked currents was significantly larger in SNC neurons compared to VTA neurons ($P = 0.000006$, $F_{(1,108)} = 22.58$, two-way ANOVA, followed by Sidak multiple comparison tests). The same data in Figs. 4A & 4B are rearranged in Figs. 4C & 4D in order to compare currents in the presence and absence of A69662 in SNC (Fig. 4C) and VTA (Fig. 4D) neurons. As shown in Figs. 4C and 4D, A769662 caused significant augmentations of diazoxide-evoked currents in SNC neurons ($P = 0.003$, $F_{(1,105)} = 8.961$, two-way ANOVA, followed by Sidak multiple comparison tests) and in VTA neurons ($P = 0.013$, $F_{(1,140)} = 6.379$, two-way ANOVA, followed by Sidak multiple comparison tests). To further analyze the main effect of A769662 treatment in SNC and VTA principal cells, we compared control diazoxide-induced currents to those in A769662 after combining data for the 30, 60 and 90 min time points. In SNC principal cells, A769662 increased average current to $329 \pm 23\%$ ($n = 40$) of the initial diazoxide-induced current, which was significantly greater than the control average of $262 \pm 12\%$ ($n = 71$; $P = 0.007$, t test). In VTA principal cells, A769662 increased current to $206 \pm 14\%$ ($n = 74$) of the initial diazoxide-induced current, which was significantly greater than the control average of $165 \pm 9\%$ ($n = 71$; $P = 0.014$, t test). These results show that A769662 augments diazoxide-evoked currents in SNC and VTA principal neurons, and the amount of current augmentation in the VTA is comparable to that seen in the SNC.

Diazoxide actions in VTA secondary neurons

Diazoxide was also tested on VTA secondary (presumed non-dopamine) neurons, which were identified by their lack of response to dopamine, little or no H-current, and relatively narrow spike width (Johnson & North, 1992). As used in the analysis of principal cells, responses to diazoxide were divided into three categories: 1) “no response” was defined as having less than 10 pA of outward current in response to diazoxide, 2) “no potentiation” was defined as having outward current of at least 10 pA but current increased by less than 50% within one hour, or 3) “potentiation” as defined by diazoxide-evoked current that increased by at least 50% during the first 60 min after beginning recording. As seen in Fig. 5A, only 1 of 10 secondary cells showed potentiation of diazoxide-evoked current over 1 hour of recording, and 4 of 10 had no response. Although 40% of secondary VTA cells had no response to diazoxide, this was not statistically different from the incidence of non-responders in VTA principal cells ($P = 0.435$, Fisher’s Exact test). When recording with A769662 in pipettes, Fig. 5B shows that 2 of 7 cells showed potentiation of diazoxide-

induced current over one hour, whereas only 1 neuron did not respond to diazoxide. Although a higher percentage of cells responded to diazoxide with A769662, this was not statistically significant from the control response ($P = 0.338$, Fisher's Exact test). It should be noted that one secondary cell showed marked potentiation of diazoxide-induced current in A769662 (an increase from 82 to 274 pA). However, this outlier may be due to the known cellular heterogeneity within the secondary cell category. Overall, the increase in current in A769662 was not statistically significant amongst secondary cells ($P = 0.356$, paired t test). These results show that a large percentage of secondary cells are unresponsive to diazoxide. Moreover, K-ATP currents in most VTA secondary cells are not augmented by the AMPK activator A769662.

Discussion

Results of our studies show that there are many similarities between SNC and VTA principal neurons in their responses to diazoxide and AMPK activation. The majority of principal neurons in both regions responded to diazoxide with outward current, which tends to increase in magnitude over the duration of whole-cell recording. Moreover, this augmentation of current was increased by the AMPK activator A769662 and diminished by the AMPK inhibitor dorsomorphin. However, significant differences also exist between VTA and SNC. Presumed dopamine neurons in the VTA were more likely than SNC neurons to be unresponsive to diazoxide, and those that responded were less likely to show augmentation of current over time. Furthermore, diazoxide-induced current in VTA neurons was significantly smaller than that in SNC neurons, both under the control condition and in the presence of the AMPK activator. These results suggest that K-ATP channels may be less widely expressed in the VTA compared to the SNC, which agrees with results of others (Liss *et al.*, 2005). Variability in diazoxide responses in VTA may be due to the fact that the VTA is comprised of subpopulations of neuron with synaptic inputs and outputs that are more heterogeneous than those in the SNC (Lammel *et al.*, 2014). However, results also suggest that AMPK activation is capable of increasing the function of K-ATP channels in a majority of VTA dopamine neurons. Consequently, K-ATP currents and their regulation by AMPK should be expected to play significant roles in regulating the excitability of VTA principal neurons.

Presumed non-dopamine (secondary) neurons in the VTA differed from principal neurons in their responses to diazoxide. In contrast to principal neurons, diazoxide-induced currents in secondary neurons did not significantly increase over time either in the presence or absence of A769662. Also unlike principal cells, A769662 did not increase the incidence of diazoxide responsiveness in secondary VTA cells. It should be noted, however, that the low number of secondary neurons recorded is a limitation of our study, and a different result might be obtained with greater numbers. Nevertheless, these findings suggest that the functional expression of K-ATP channels and its regulation by AMPK differ according to cell type, and this is despite the fact that K-ATP channels and AMPK are widely expressed in virtually all regions of the brain (Dunn-Meynell *et al.*, 1998; Turnley *et al.*, 1999; Culmsee *et al.*, 2001; Zhou *et al.*, 2012). This point is reinforced by our own studies in STN neurons in which we showed that diazoxide-induced currents do not augment with time during whole-cell recordings, nor are they potentiated by AMPK activators (Shen *et al.*,

2014). Moreover, we showed that calcium-dependent K-ATP currents can be evoked by glutamate agonists in STN neurons but not in SNC principal neurons or in SNR neurons (Shen & Johnson, 2010; Shen & Johnson, 2013). These results clearly demonstrate that K-ATP currents and their regulation by second messenger systems differ significantly according to neuronal type.

The question remains as to how the process of whole-cell recording triggers the potentiation of K-ATP currents in presumed dopamine neurons. The fact that dorsomorphin effectively suppressed the potentiation of diazoxide currents suggests that augmentation of currents is mediated by AMPK activation. Our previous studies in SNC neurons showed that it is the duration of whole-cell recording rather than previous exposure to diazoxide that is critical in causing potentiation of diazoxide-induced currents (Shen *et al.*, 2016). The result in the present study showing that current evoked by tolbutamide does not change over time in the absence of diazoxide suggests that whole-cell recording does not cause spontaneous opening of these channels but rather increases the magnitude of K-ATP current that can be generated. As a consequence of intracellular dialysis with pipette contents, disturbance of calcium homeostasis is a possible mechanism that may potentiate K-ATP current, and calcium-dependent activation of AMPK is well established (Carling *et al.*, 2008). Alterations in phosphoinositide levels in lipid membranes may also alter the function of K-ATP channels (Krauter *et al.*, 2001). Because our pipette solutions contained ATP, activation of AMPK by intracellular metabolism of ATP to AMP is also possible. As a consequence of AMPK activation, this may increase the translocation of K-ATP channels to the cell surface as has been shown in pancreatic beta-cells (Chen *et al.*, 2013; Park *et al.*, 2013). Studies in progress are designed to define the mechanism by which K-ATP currents are potentiated in SNC principal neurons.

It should be noted that diazoxide is known to have multiple actions in addition to its effect on K-ATP channels that could be important for our study (Coetzee, 2013). Concentrations of diazoxide similar to that used in our study have been reported to activate protein kinase C (PKC) in cardiac myocytes, and this may indirectly activate mitochondrial K-ATP channels (Kim *et al.*, 2006). Furthermore, PKC has been shown to activate AMPK in monocytes (Chang *et al.*, 2012). However, PKC has been shown to reduce the activity of K-ATP channels in hippocampal cells (Hu *et al.*, 2003) and HEK-293 cells (Manna *et al.*, 2010). Moreover, our previous work in SNC neurons showed that the gradual increase in K-ATP current is a function of the duration of whole-cell recording and is not dependent upon previous diazoxide exposure (Shen *et al.*, 2016). Nevertheless, our lab plans further work to investigate the possible roles of PKC and other second messenger systems in the regulation of K-ATP current in dopamine neurons.

Although K-ATP currents are less pronounced in VTA compared to SNC principal neurons, there are important functional consequences to these currents. K-ATP currents provide an inhibitory influence on neuronal excitability and in this capacity would be predicted to restrain dopamine release from VTA-innervated nuclei. As part of the mesolimbic system, reduced dopamine release would be expected to dampen dopamine-mediated behaviors that affect motivation, behavioral reward, and possibly reinforcement important in drug abuse (Le Moal & Simon, 1991; Grace *et al.*, 2007). Although brief activation of K-ATP channels

can provide neuroprotection (Tai & Truong, 2002; Yoshida *et al.*, 2012), prolonged K-ATP channel activation has been linked to increased risk of cell death (Liss *et al.*, 2005; Toulorge *et al.*, 2010). Because K-ATP channels appear to be functionally up-regulated in SNC neurons compared to VTA neurons, this could help explain why VTA neurons are relatively spared compared to the destruction of SNC neurons in a degenerative disease such as Parkinson's disease (German *et al.*, 1992; McRitchie & Halliday, 1995; Dragicevic *et al.*, 2014). We suggest that the levels of K-ATP channel expression in the VTA can have important implications for dopamine-dependent behaviors as well as resistance to potential neurotoxicity.

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Abbreviations

AMPK	5'-adenosine monophosphate-activated protein kinase
K-ATP channel	ATP-sensitive K ⁺ channel
Kir channel	inwardly rectifying K ⁺ channel
SNC	substantia nigra zona compacta
SNR	substantia nigra zona reticulata
STN	subthalamic nucleus
SUR	sulfonylurea receptor
VTA	ventral tegmental area

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Rat midbrain slices were superfused with the K-ATP opener diazoxide every 30 min while recording from principal neurons in the SNC and VTA using whole-cell patch pipettes. Although the AMP kinase activator A769662 augmented diazoxide-induced currents in both cell types, currents were larger in SNC neurons. K-ATP channels and their regulation by AMP kinase may play important roles in regulating SNC and VTA dopamine neuron physiology.

Fig. 1.

Diazoxide evokes progressively larger outward currents in presumed dopamine neurons. A) Diazoxide (200 μ M), applied by superfusion, evokes currents that increase in magnitude over the duration of whole-cell recording in an SNC principal neuron. B) Tolbutamide (100 μ M) causes nearly complete block of diazoxide-induced currents in a VTA principal neuron. C) The effect of the third application of diazoxide is completely blocked by tolbutamide in this SNC principal cell.

Fig. 2.

Responses to diazoxide in SNC and VTA principal neurons in the presence and absence of A769662. Responses were categorized as either having “no response” (less than 10 pA of outward current), outward current greater than 10 pA but “no potentiation” over one hour, or current that showed “potentiation” as defined by at least a 50% increase in current over one hour of recording. Responses are illustrated for SNC control neurons (A), VTA control neurons (B), SNC neurons in A769662 (C) and VTA neurons in A769662 (D). Numbers in parentheses represent number of neurons in each category. VTA neurons were significantly more likely to have no response to diazoxide compared to SNC neurons, but responsiveness of VTA neurons to diazoxide was significantly increased by A769662.

Fig 3.

Dorsomorphin inhibits diazoxide-induced current in VTA principal neurons. Data represent currents evoked by the second (before dorsomorphin) and third (during dorsomorphin) applications of diazoxide (200 μ M). Dorsomorphin superfusion was begun 10 min before the third application of diazoxide, and data were normalized to the amplitude current evoked by the first diazoxide application. These paired data are from five individual neurons.

Fig. 4.

Augmentation of diazoxide currents in SNC and VTA dopamine neurons in the presence and absence of A769662. Data are expressed as percent of initial diazoxide current as calculated for each neuron. Graphs show percent of initial diazoxide current in SNC and VTA control neurons (A) and when recorded with A769662 in pipettes (B). These same data are presented in C & D to show percent of initial diazoxide currents in the presence and absence of A769662 for SNC neurons (C) and VTA neurons (D). VTA neurons that were unresponsive to diazoxide were omitted. Each data point represents the average responses of 10–28 neurons. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; two-way ANOVA followed by Sidak multiple pairwise comparisons).

Fig. 5.

Responses to diazoxide in VTA secondary neurons in the presence and absence of A769662. Responses were categorized as either having “no response” (less than 10 pA of outward current), outward current greater than 10 pA but “no potentiation” over one hour, or current that showed “potentiation” as defined by at least a 50% increase in current over one hour of recording. Responses are illustrated for secondary neurons in the control condition (A) and when recorded with A769662 in pipettes (B). Numbers in parentheses represent number of neurons in each category. A769662 had no significant effect on either the percentage of cells responding to diazoxide or having their response to diazoxide potentiated.