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Dopamine evokes a trace amine receptor-dependent inward current that is regulated by AMP kinase in substantia nigra dopamine neurons

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Abstract

We reported recently that activators of AMP-activated protein kinase (AMPK) slow the rundown of current evoked by the D2 autoreceptor agonist quinpirole in rat substantia nigra compacta (SNC) dopamine neurons. The present study examined the effect of AMPK on current generated by dopamine, which unlike quinpirole, is a substrate for the dopamine transporter (DAT). Using whole-cell patch-clamp, we constructed current-voltage (I-V) plots while superfusing brain slices with dopamine (100 μ M) for 25 min. Two minutes after starting superfusion, dopamine evoked a peak current with an average slope conductance of 0.97 nS and an estimated reversal potential (E_{rev}) of -113 mV, which is near that expected for K^+ . But after 10 min of superfusion, dopamine-evoked currents had shifted to more depolarized values with a slope conductance of 0.64 nS and an E_{rev} of -83 mV. This inward shift in current was completely blocked by the DAT inhibitor GBR12935. However, an AMPK blocking agent (dorsomorphin) permitted the emergence of inward current despite the continued presence of the DAT inhibitor. When D2 autoreceptors were blocked by sulpiride, I-V plots showed that dopamine evoked an inward current with an estimated slope conductance of 0.45 nS with an E_{rev} of -57 mV. Moreover, this inward current was completely blocked by the trace amine-associated receptor 1 (TAAR1) antagonist EPPTB. These results suggest that dopamine activates a TAAR1-dependent non-selective cation current that is regulated by AMPK.

Graphical Abstract

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Trace amine receptor antagonist blocks dopamine-induced inward current

Keywords

AMP kinase; dopamine; patch-clamp; brain slice; dopamine transporter; trace amine

INTRODUCTION

Dopamine output from the substantia nigra zona compacta (SNc) has many important effects on behavior, such as enabling movement, habit formation, facilitating learning, and participating in behavioral reward (Schultz, 1998; Yin and Knowlton, 2006; Albin et al., 1995). In addition, dopamine output from the ventral tegmental area (VTA) is pivotal in mediating the rewarding aspects of drugs of abuse (Bonci et al., 2003; Di Chiara and Imperato, 1988). Given the significance of dopamine neurotransmission on behavior, a better understanding of how dopamine neuronal excitability is regulated has clear importance. One important regulator of excitability is the D2 autoreceptor that is expressed on the somata and dendrites of dopamine neurons (Cheramy et al., 1981; Uchida et al., 2000). Because the D2 autoreceptor evokes a G protein-coupled inwardly rectifying K⁺ (GIRK) channel, it suppresses neuronal excitability and inhibits further dopamine output. However, intense or prolonged stimulation of D2 autoreceptors can cause these receptors to desensitize and thereby disinhibit neuronal activity (Bartlett et al., 2005). Dopamine D2 autoreceptor desensitization can thereby facilitate further dopamine output and has been implicated as a mechanism that contributes to behavioral sensitization to drugs of abuse (Madhavan et al., 2013).

Studies in our lab have recently focused on the effects of AMP-activated protein kinase (AMPK) on dopamine neuronal excitability. AMPK is a master enzyme complex that is involved in maintaining energy homeostasis (Hardie et al., 2012). A heterotrimeric complex, AMPK is activated by conditions of energy depletion in which levels of AMP rise and ATP fall (Gowans et al., 2013). After binding to AMP, AMPK is activated by phosphorylation by upstream kinases (Carling et al., 2008). Consistent with its role to maintain energy homeostasis, AMPK acts to limit ATP use in somatic tissues by reducing gluconeogenesis and limiting synthesis of protein and lipids, while boosting ATP synthesis by mitochondria (Hardie et al., 2003; Qi and Young, 2015). In neurons, a major cause of energy utilization

involves the ATP-dependent removal of Na^+ and Ca^{2+} that enter the cell during membrane depolarization (Powis et al., 1983; Mata et al., 1980; Khatri and Man, 2013). Therefore, we hypothesize that AMPK activation might conserve energy in neurons by reinforcing mechanisms that reduce neuronal excitability. Because the D2 autoreceptor represents an important mechanism for reducing dopamine neuronal excitability, our lab has been interested in evaluating the hypothesis that AMPK might augment the function of the D2 autoreceptor.

We reported recently that AMPK activators slowed the desensitization of D2 autoreceptors in SNC dopamine neurons (Yang et al., 2019). While superfusing rat brain slices with a desensitizing concentration of the D2 agonist quinpirole, we found that an AMPK activator greatly slowed the rundown of quinpirole-induced current. However, the action of AMPK required the presence of a D1-like receptor agonist, and the effect of AMPK was mediated by the cAMP/PKA pathway. As expected, the slowing of current rundown by AMPK activation was completely blocked by a D1-like antagonist. Figure 1 summarizes findings of our previous work regarding the pathways by which AMPK slows D2 autoreceptor desensitization.

In the present study, we examined the effects of dopamine in an effort to better understand factors that influence the rundown of current induced by this endogenous neurotransmitter. Using whole-cell patch-clamp recordings in SNC dopamine neurons in slices of rat midbrain, we studied effects of these agents on the rundown of outward current evoked by prolonged superfusion of dopamine. In addition to the outward current that is typically generated by stimulation of D2 autoreceptors, dopamine also evoked an inward current that developed after 10 min of superfusion. This inward current was blocked by a DAT inhibitor, but could be re-established by adding an AMPK blocker to the superfusate. The inward current was blocked by the trace amine receptor antagonist EPPTB, suggesting that the inward current was generated by the trace amine-associated receptor 1 (TAAR1). These results show that an excitatory inward current can be generated by dopamine, which would be expected to oppose the inhibitory influence of D2 autoreceptors.

EXPERIMENTAL PROCEDURES

Animals and slice preparation

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. Sprague Dawley rat pups (11–23 days post-natal) were obtained from Envigo, USA. Protocols were approved and reviewed by the animal care committee at the Veterans Affairs Portland Health Care System (Oregon, USA). A total of 165 animals were used in our studies.

Rat pups were anesthetized with isoflurane (Baxter, USA) and euthanized by rupture of major thoracic vessels. The brain was removed quickly and submerged in ice-cold sucrose cutting solution made of (in mM) sucrose (196), KCl (2.5), MgCl_2 (3.5), CaCl_2 (0.5), NaH_2PO_4 (1.2), NaHCO_3 (26), and D-glucose (20) that had been bubbled with 95%/5% O_2/CO_2 blended (carbogen) gas. A brain block containing the ventral midbrain was mounted

on a platform with cyanoacrylate glue and horizontal slices (250 μm thick) containing the substantia nigra were cut with a vibrating microtome (Leica Biosystems, USA) while submerged in chilled cutting solution bubbled with carbogen. Slices were placed in a holding chamber containing artificial cerebral spinal fluid (aCSF) saturated with carbogen gas at room temperature. The aCSF was composed of (in mM): NaCl (126), KCl (2.5), CaCl_2 (2.4), MgCl_2 (1.2), NaH_2PO_4 (1.2), NaHCO_3 (19), and D-glucose (11).

Electrophysiology

After at least one hour in the holding chamber, a hemi-slice containing the SNC was transferred into a submersion recording chamber mounted on an upright microscope (Zeiss Microscopy, USA) and held down with platinum wire pieces and perfused continuously with warmed (35°C) aCSF flowing at 2 mL/min. Using bright field optics, slices were visualized under a 5x objective (50x final magnification) to initially center the microscope over the SNC. Presumed dopamine neurons in the SNC were recorded in the region immediately rostral to the medial terminal nucleus of the accessory optic tract (Paxinos and Watson, 1986). Microscope output was captured with a digital camera (Orca 5G, Hamamatsu, Japan) and projected onto a computer monitor.

Patch pipettes were pulled from thick-walled 1.5 mm borosilicate glass capillary tubing (World Precision Instruments, USA) on a vertical microelectrode puller (PC 10, Narishige Instrument Co, Japan). Immediately before use, the pipettes were backfilled with chilled intracellular solution (pH 7.3) containing (in mM): potassium gluconate (138), MgCl_2 (2), CaCl_2 (1), EGTA (11), HEPES (10), ATP (1.5), and GTP (0.3). The ATP and GTP salts were added to a thawed aliquot of internal solution stock at the start of each week of experiments.

Pipette positioning onto neurons was visualized with a submersible 40x objective (400x final magnification) using differential interference contrast optics under infrared illumination. Currents were recorded from single SNC neurons under voltage clamp (−60 mV) using patch pipettes (3–6 M Ω) in whole-cell configuration. Current was filtered (2 kHz low pass) and amplified (Axopatch 1D, Molecular Devices, USA), digitized (Digidata 1550, Molecular Devices, USA) and saved to computer storage with digitizer associated software (Clampex v10, Molecular Devices, USA). Presumed dopamine-containing neurons in the SNC were identified by having relatively broad (1–2 ms) spontaneous action currents and large hyperpolarization-induced inward currents (Lacey et al., 1989). Recordings were corrected for a liquid junction potential offset of 10 mV.

Current-voltage plots

Membrane voltage was held at −120 mV for 0.5 sec before initiating a depolarizing voltage ramp from −120 to −60 mV over 600 msec. Slices were superfused with ZD7288 (50 μM) 15 min before the ramp in order to block H-current (Harris et al., 1994). Ramps were conducted before dopamine, and at 2 min (peak effect), 10 min, and 20 min after starting superfusion with dopamine. Current traces were smoothed prior to data analysis by taking the average of every 30 samples.

Drugs and chemicals

All drugs were dissolved in either purified water or dimethyl sulfoxide (DMSO). Stock solutions containing DMSO were made such that the final concentration in slice superfusate was diluted by at least 1000-fold. A769662, dorsomorphin dihydrochloride (compound C), STO609 acetate, and ZD7288 were purchased from Cayman Chemical (USA). Dopamine HCl, GBR12935, cocaine HCl, (–) quinpirole HCl, and (–)-sulpiride were purchased from Sigma–Aldrich (USA). SCH39166 was obtained from Tocris/Bio-technie (UK), and EPPTB (N-(3-ethoxyphenyl)-4-pyrrolidin-1-yl-3-trifluoromethyl-benzamide) was a generous gift from Dr. Aaron Janowsky at VA Portland Health Care System, Portland, OR. A769662 was dissolved to working strength in internal pipette solutions; this agent diffused passively from pipette solutions into the cytosol during whole cell recordings. All other drugs were dissolved to final concentration in aCSF and added to the brain slice superfusate. Typically 60 sec were required for solution changes to traverse the tubing system to show initial effect at the neuron. Dopamine solutions were made daily and kept on ice to retard oxidation.

Data measurement and analysis

Slices were superfused continuously with either dopamine or quinpirole for 25 min, and currents (at –60 mV) were measured at 2 min (peak current) and at 5, 10, 20 and 25 min after starting superfusion. Sulpiride was added to the superfusate at the 20 min time point to measure the amplitude of current blocked by sulpiride (sulpiride-sensitive current). Decay plots of dopamine- or quinpirole-induced current were analyzed at the 5, 10 and 20 min time points using a linear mixed model with the following parameters: treatment and time were fixed effects (sum of squares method III), and repeated measures, from each neuron, were modeled as repeated within-subject, using first order autoregressive covariance structure (AR1). The restricted maximum likelihood estimator was used. Post hoc pairwise comparisons of estimated means were adjusted using Sidak's method. Normality of linear mixed model datasets were inspected on Studentized residual plots. Data points that exceeded three standard deviations were excluded. Between group analysis of currents blocked by sulpiride were done using either Student's or Welch's unpaired *t* tests, depending upon equal versus unequal variance as determined by Levene's test.

Current-voltage (I-V) plots for dopamine-induced currents were constructed by subtracting currents recorded during dopamine superfusion from those recorded before superfusion. Linear mixed modeling was used to analyze within-subject differences in ramp currents within a treatment group, using voltage and elapsed time as fixed factors. Slope conductance and reversal potential (E_{rev}) were estimated for each neuron using linear regression. Differences in conductance and E_{rev} were tested using paired Student *t* tests. Statistics were computed with SPSS v25 (IBM North America, USA) or R v3.6.0 (R Foundation for Statistical Computing, Australia). Numerical data in the text and error bars in figures are expressed as mean average $\pm$ standard error of the mean. Significance was accepted with $P < 0.05$.

RESULTS

AMPK activator slows rundown of dopamine-evoked current

Effects of the AMPK activator A769662 on currents evoked by dopamine are shown in Fig. 2. Slices were superfused with a relatively high concentration of dopamine (100 μ M) that was intended to induce autoreceptor desensitization. Dopamine was superfused continuously for 25 min, and sulpiride (1 μ M) was added to the superfusate during the last 5 min in order to measure the magnitude of any residual current mediated by D2 autoreceptors. As shown in the upper current trace in Fig. 2A, dopamine evoked an outward current (at -60 mV) that decayed to zero over the next 5–10 min, and sulpiride had no significant effect during the last 5 min of the recording. However, the middle trace in Fig. 2A shows that the rundown of dopamine-induced current was markedly slowed when the pipette solution contained A769662 (10 μ M). Moreover, sulpiride caused an inward current when recording with A769662, which suggests that a significant amount of D2 autoreceptor-mediated current remained after superfusing dopamine for 20 min. Because we showed previously that a D1-like antagonist completely blocked the effect of A769662 on quinpirole-evoked current (Yang et al., 2019), we proceeded to test the effect of the D1 antagonist SCH39166 on the ability of A769662 to slow rundown of dopamine-evoked current. In this experiment, the slice was superfused continuously with SCH39166 (1 μ M) beginning 10 min before dopamine. But as seen in the lower trace in Fig. 2A, the D1-like antagonist caused only a partial block of the effect of A769662 on rundown of dopamine-induced current. Because SCH39166 is known to have micromolar affinity for the D2 receptor (Tice et al., 1994), it is important to note that SCH39166 did not diminish the peak effect of dopamine (see Fig. 2B). This shows that SCH39166 has no significant effect on D2 autoreceptor-mediated current in our studies.

Effects of A769662 and SCH39166 on dopamine-induced current are summarized in the current decay plot in Fig. 2B. As we reported previously, A769662 caused a significant slowing of the rundown of current evoked by dopamine (Yang et al., 2019). Mixed model analysis showed there was a significant effect of A769662 on dopamine-induced current ($F_{(1,42.56)} = 91.41$, $P < 0.0001$). Also shown in Fig. 2B, this effect of A769662 was reduced significantly by SCH39166, but it did not completely block the effect. Mixed model analysis showed there was a significant effect of SCH39166 on dopamine-induced current in the presence of A769662 ($F_{(1,31.18)} = 6.012$, $P < 0.05$). However, the dopamine-induced current decay with A769662 plus SCH39166 was still significantly different from the dopamine control plot ($F_{(1,27.10)} = 18.75$, $P < 0.001$). Figure 2B also shows that the addition of SCH39166 alone had no significant effect on rundown of current evoked by dopamine ($F_{(1,26.86)} = 0.018$, $P = 0.894$).

Effects of SCH39166 on sulpiride-sensitive currents evoked by dopamine are shown in Fig. 2C. In the continued presence of dopamine, sulpiride-sensitive current in A769662 (16.3 ± 3.1 pA, $n = 14$) was significantly greater than that evoked by dopamine in the control condition (0.6 ± 0.9 pA, $n = 14$; $t_{(15.31)} = 4.928$, $P < 0.001$, Welch's t test). Although mixed model analysis showed that SCH39166 had a significant effect on current decay in the presence of A769662, sulpiride-sensitive current in SCH39166 (8.0 ± 3.7 pA, $n = 9$) was not

significantly different from that recorded in A769662 without SCH39166 ($t(21) = 1.719$, $P = 0.100$, Student's t test). Sulpiride-sensitive current in A769662 plus SCH39166 was also not significantly different from that recorded for dopamine plus SCH39166 ($t(8.98) = 1.930$, $P = 0.086$, Welch's t test). Taken together, these results suggest that a D1-like antagonist reduces but does not completely block the ability of an AMPK activator to slow the rundown of dopamine-induced current. These results raised the possibility that multiple mechanisms might contribute to the ability of AMPK to slow the rundown of dopamine-induced current.

Dorsomorphin blocks the combined effects of A769662 and GBR12935 on dopamine-induced current

Dopamine, unlike synthetic dopamine agonists such as quinpirole, is a substrate for the dopamine transporter (DAT). Therefore, we considered the possibility that AMPK might slow the apparent rundown of dopamine-induced current by inhibiting DAT. We proceeded to test whether or not a DAT inhibitor could mimic the effect of an AMPK activator on rundown of dopamine-induced current. SNC neurons were recorded with pipettes that contained the AMPK activator A769662 (10 μM), whereas the DAT inhibitor GBR12935 (1 μM) was added to the superfusate 10 min before starting dopamine superfusion. Superfusate also contained SCH39166 (1 μM) in order to block possible stimulation of D1-like receptors. The current decay graph in Fig. 3A shows that the addition of GBR12935 to A769662 did not cause a further slowing of dopamine-induced current rundown compared to A769662 alone ($F_{(1,12.32)} = 3.736$, $P = 0.077$, mixed model analysis). We subsequently tested the effect of the AMPK inhibitor dorsomorphin (30 μM), which inhibits AMPK activity by blocking phosphorylation of Thr-172 by upstream kinases (Kunanusornchqi et al., 2016; Zhou et al., 2001). As shown in Fig. 3A, dorsomorphin, which was added to the superfusate simultaneously with GBR12935, completely blocked the effects of both A769662 and GBR12935 on dopamine-induced current. Mixed model analysis showed there was a significant effect of dorsomorphin on dopamine-induced current in the presence of A769662 plus GBR12935 ($F_{(1,8.64)} = 42.50$, $P < 0.001$). Moreover, the rundown of dopamine-induced current in A769662, GBR12935 and dorsomorphin was not significantly different from that recorded in the dopamine control condition ($F_{(1,22.02)} = 0.195$, $P = 0.662$).

Figure 3B shows the effects of GBR12935, A769662 and dorsomorphin on sulpiride-sensitive currents. Sulpiride-sensitive current in GBR12935 plus A769662 (21.0 ± 2.5 pA, $n = 5$) was significantly greater than that recorded in A769662 alone ($t(12) = 2.400$, $P < 0.05$, Student's t test). Moreover, sulpiride-sensitive current recorded in dorsomorphin plus A769662 and GBR12935 (-0.3 ± 0.3 pA, $n = 4$) was significantly different from that recorded without dorsomorphin ($t(4.09) = 8.317$, $P < 0.01$, Welch's t test). These results suggest that a DAT inhibitor and an AMPK activator have an additive effect on residual sulpiride-sensitive current evoked by dopamine. Moreover, these data suggest that an AMPK inhibitor can completely block the combined effects of an AMPK activator and a DAT inhibitor.

AMPK inhibitors prevent the effect of GBR12935 on dopamine-induced current

Having found that dorsomorphin blocked the effects of GBR12935 plus A769662 on current rundown, we proceeded to examine the effect of a DAT inhibitor in the absence of the AMPK activator. The current trace in Fig. 4A shows the typically rapid rundown of dopamine-induced current under control conditions. In contrast, the current trace in Fig. 4B shows that GBR12935 (1 μ M), which was added to the superfusate 10 min before dopamine, caused a marked slowing of current rundown. Moreover, this trace shows that sulpiride caused an inward shift in holding current in the presence of GBR12935. Finally, the trace in Fig. 4C shows that the slowing of dopamine-induced current rundown by GBR12935 was prevented when the slice was superfused with dorsomorphin plus GBR12935.

A summary of these data is shown in Fig. 5A. Dorsomorphin completely blocked the ability of GBR12935 to slow the rundown of dopamine-induced current. Mixed model analysis showed there was a significant effect of dorsomorphin on dopamine-induced current in the presence of GBR12935 ($F_{(1,14.61)} = 16.597$, $P < 0.01$). We also examined the effect of STO609, which blocks the ability of the upstream kinase CaMKK β to phosphorylate Thr-172 and thereby prevents AMPK activation (Hawley et al., 2005; Tokumitsu et al., 2002). Superfusion with STO609 plus GBR12935 was started 10 min before dopamine and continued throughout the recording. As shown in Fig. 5A, STO609 (10 μ M) also completely prevented the ability of GBR12935 to slow the rundown of dopamine-induced current. Mixed model analysis showed there was a significant effect of STO609 on dopamine-induced current in the presence of GBR12935 ($F_{(1,13.96)} = 26.011$, $P < 0.001$). It should be noted that we showed previously that dorsomorphin and STO609, when superfused alone, had no effect on membrane current in SNC neurons (Yang et al., 2019).

Data showing the effects of GBR12935, dorsomorphin, and STO609 on sulpiride-sensitive currents are shown in Fig. 5B. Sulpiride-sensitive current in GBR12935 (20.4 ± 4.6 pA, $n = 6$) was significantly greater than that recorded in dopamine alone ($t(5.40) = 4.212$, $P < 0.01$, Welch's t test). Sulpiride-sensitive currents in GBR12935 plus dorsomorphin (0.1 ± 0.1 pA, $n = 6$) or STO609 (0.5 ± 0.2 pA, $n = 6$) were both significantly smaller compared to GBR12935 alone ($t(5.01) = 4.397$, $P < 0.01$ and $t(5.01) = 4.311$, $P < 0.01$, respectively). Taken together, these data support the conclusion that AMPK blocking agents prevent the ability of a DAT uptake inhibitor to slow the rundown of dopamine-induced current.

Dorsomorphin blocks the effect of cocaine on rundown of dopamine current

To examine whether or not the interaction between AMPK blocking agents and DAT inhibition was unique to GBR12935, we next investigated the effect of dorsomorphin on the ability of cocaine to slow the rundown of dopamine-induced current. Superfusion with cocaine (10 μ M) was begun 10 min before dopamine and continued throughout experiments. As shown in Fig. 6A, cocaine caused marked slowing of the rundown of current evoked by dopamine. Mixed model analysis showed there was a significant effect of cocaine on dopamine-induced current ($F_{(1,22.49)} = 43.276$, $P < 0.0001$). Figure 6A also shows that superfusion with dorsomorphin (30 μ M), which was begun at the same time as cocaine, interfered with the ability of cocaine to slow current rundown. Mixed model analysis showed there was a significant effect of dorsomorphin on dopamine-induced current in the presence

of cocaine ($F_{(1,10.35)} = 10.366$, $P < 0.01$). However, dorsomorphin did not completely block the effect of cocaine, as the current rundown plot for cocaine plus dorsomorphin remained significantly different from that for dopamine alone ($F_{(1,25.14)} = 8.992$, $P < 0.01$, mixed model analysis).

Data showing effects of dorsomorphin and cocaine on sulpiride-sensitive currents are shown in Fig. 6B. Sulpiride-sensitive current recorded in cocaine (20.2 ± 5.4 pA, $n = 5$) was significantly greater than that recorded for dopamine alone ($t(4.23) = 3.547$, $P < 0.05$, Welch's t test). However, sulpiride-sensitive currents in cocaine plus dorsomorphin (0.4 ± 0.6 pA, $n = 5$) was significantly smaller compared to cocaine ($t(4.11) = 3.614$, $P < 0.05$, Welch's t test). These data show that an AMPK blocking agent can reduce the ability of two different DAT inhibitors to slow the rundown of dopamine-induced current.

Dorsomorphin does not alter current evoked by quinpirole

We considered the possibility that an AMPK blocking agent might reduce the effect of a DAT inhibitor by facilitating the desensitization of D2 autoreceptors. Therefore, we investigated the effect of dorsomorphin on current evoked by quinpirole, a D2 receptor agonist that is not a substrate for DAT (Bolan et al., 2007; Ledonne et al., 2010). We combined quinpirole with the D1-like agonist SKF38393 in order to mimic the ability of dopamine to stimulate both D1- and D2-like receptors. Quinpirole (30 μ M) and SKF38393 (10 μ M) were superfused for 25 min, and sulpiride (10 μ M) was added during the last 5 min as we described previously (Yang et al., 2019). As shown in Fig. 7A, pretreatment with dorsomorphin had no effect on the rundown of quinpirole-induced current ($F_{(1,16.75)} = 0.149$, $P = 0.704$, mixed model analysis). Similarly, Fig. 7B shows that sulpiride-sensitive current evoked by quinpirole plus SKF38393 (13.5 ± 3.3 pA, $n = 11$) was not significantly altered by the addition of dorsomorphin (18.0 ± 6.9 pA, $n = 5$; $t(4.64) = 0.626$, $P = 0.561$, Welch's t test). These results show that dorsomorphin does not accelerate the desensitization of D2 autoreceptors.

DAT inhibition blocks inward current evoked by dopamine

Results of the above experiments clearly show that AMPK inhibitors antagonize the ability of dopamine uptake blockers to slow rundown of dopamine-induced current. In order to investigate underlying mechanisms, we proceeded to examine the voltage-dependence of currents evoked by dopamine in the presence and absence of a DAT inhibitor. Currents were recorded during voltage ramps from -120 to -60 mV over 600 msec, and slices were superfused with ZD7288 (50 μ M) 15 min before the ramp in order to block H-current (Harris et al., 1994). Ramps were conducted before dopamine, and at 2 min (peak effect), 10 min and 20 min after starting dopamine superfusion. Figure 8A shows raw current traces recorded during voltage ramps. One can see that dopamine evoked a peak outward current with an E_{rev} that was near that expected for K^+ as predicted by the Nernst equation (-106 mV). However, currents recorded after 10 and 20 min of dopamine superfusion reveal an inward shift in I-V plots. These results are summarized in Fig. 8B, which shows "net" dopamine-induced currents that were calculated by subtracting currents recorded in dopamine from those recorded before dopamine (control). Mixed model analysis showed that currents recorded after 10 min of dopamine were significantly different from those

recorded at the peak (2 min) dopamine effect ($F_{(1,60)} = 39.30$, $P < 0.0001$). Dopamine-induced slope conductance and E_{rev} were calculated for each neuron via linear regression. The average conductance increase produced by dopamine at 10 min (0.64 ± 0.19 nS) was significantly smaller than that evoked at 2 min (0.97 ± 0.14 nS, $n = 7$; $t(6) = 3.562$, $P < 0.01$, paired t test). Moreover, the estimated E_{rev} for dopamine-induced current at 10 min (-83 ± 5 mV) was significantly more depolarized than that estimated at 2 min (-113 ± 6 mV, $n = 7$; $t(6) = 3.562$, $P < 0.05$, paired t test). These data suggest that an inward current develops slowly during continuous application of dopamine.

Figure 8C shows I-V relationships for subtracted (net) dopamine-induced currents in the presence of GBR12935 (1 μ M). Mixed model analysis showed that currents recorded after 10 min of dopamine plus GBR12935 were not significantly different from those recorded at peak (2 min) dopamine effect ($F_{(1,82)} = 2.99$, $P = 0.088$). Furthermore, the E_{rev} for dopamine-induced current at 2 min (-111 ± 3 mV) was not significantly different from that estimated at 10 min (-114 ± 6 mV, $n = 8$; $t(7) = 0.599$, $P = 0.568$, paired t test). The E_{rev} at 20 min (-112 ± 14 mV, $n = 6$) was also not different from that recorded at 2 min ($t(5) = 0.197$, $P = 0.862$, paired t test). However, the average conductance increase produced by dopamine at 10 min (0.77 ± 0.08 nS) was significantly smaller than that evoked at 2 min (1.14 ± 0.11 nS, $n = 8$; $t(7) = 3.930$, $P < 0.01$, paired t test). The lack of change in E_{rev} suggests that the DAT inhibitor prevents the development of a dopamine-induced inward current, whereas the progressive reduction in conductance may be due to D2 autoreceptor desensitization.

Dorsomorphin prevents the effect of DAT inhibition on dopamine-induced inward current

We considered the possibility that a DAT inhibitor prevents the development of dopamine-induced inward current by blocking the sodium- and chloride-dependent current that accompanies uptake of dopamine by DAT (Mortensen and Amara, 2003). However this seemed unlikely because: 1) Peak dopamine current reversed very near that expected for K^+ , which should not be the case if there was substantial inward uptake current, and 2) Dopamine-induced inward current steadily increased over 10–20 min, whereas an uptake current would be expected to be present from the beginning. In order to better understand the interaction between AMPK and a DAT inhibitor, we constructed I-V plots for dopamine-induced currents in the presence of GBR12935 and dorsomorphin.

Figure 9A shows I-V relationships for subtracted (net) dopamine-induced currents in the presence of GBR12935 plus dorsomorphin. This summary graph shows that the presence of dorsomorphin (30 μ M) permits the development of a dopamine-induced inward current despite the continuous presence of GBR12935 (1 μ M). Mixed model analysis showed that currents recorded after 10 min of dopamine plus GBR12935 and dorsomorphin were significantly different from those recorded at peak (2 min) dopamine effect ($F_{(1,71)} = 48.63$, $P < 0.0001$). When recorded in GBR12935 plus dorsomorphin, the average conductance increase produced by dopamine at 10 min (0.52 ± 0.06 nS) was significantly smaller than that evoked at 2 min (1.08 ± 0.22 nS, $n = 8$; $t(7) = 2.599$, $P < 0.05$, paired t test). Furthermore, the E_{rev} for dopamine-induced current at 10 min (-78 ± 12 mV) was significantly more depolarized than that recorded at 2 min (-119 ± 8 mV, $n = 8$; $t(7) =$

0.6.270, $P < 0.001$, paired t test). Moreover, mixed model analysis showed that the I-V data for dopamine-alone at 10 min (shown in Fig. 5A) was not significantly different from the 10 min data recorded in GBR12935 plus dorsomorphin ($F_{(1,11.00)} = 0.000171$, $P = 0.990$). These results show that dorsomorphin completely reversed the ability of GBR12935 to suppress dopamine-induced inward current.

A TAAR1 antagonist blocks the effect of dorsomorphin in the presence of a DAT inhibitor

In addition to its ability to activate D1- and D2-like receptors, dopamine is also an agonist at the trace amine-associated receptor 1 (TAAR1) (Xie and Miller, 2007; Borowsky et al., 2001). Moreover, TAAR1 activation has been reported to increase dopamine neuronal excitability (Dave et al., 2019; Revel et al., 2012a), although others report more complex effects on membrane current (Ledonne et al., 2011). We tested for involvement of a trace amine effect by investigating the effect of EPPTB, which is a selective TAAR1 antagonist with little affinity for DAT (Bradaia et al., 2009). Slices were superfused with EPPTB (3 μ M) plus GBR12935 and dorsomorphin beginning 10 before dopamine. As shown in Fig. 9B, EPPTB completely prevented the development of dopamine-induced inward current when recorded with GBR12935 plus dorsomorphin (compare Fig. 9B to Fig. 9A). In slices pretreated with EPPTB, mixed model analysis showed that ramp currents recorded after 10 min of dopamine superfusion were not significantly different from those recorded at 2 min in the presence of GBR12935 and dorsomorphin ($F_{(1,49)} = 1.174$, $P = 0.284$). In the presence of EPPTB, dorsomorphin and GBR12935, the average dopamine conductance at 10 min (0.77 ± 0.13 nS) was significantly smaller than at 2 min (1.24 ± 0.18 nS, $n = 6$; $t(5) = 2.81$, $P < 0.05$), but here was no significant difference in E_{rev} (-111 ± 5 versus -122 ± 7 mV; $t(5) = 1.857$, $P = 0.122$). These results suggest that the dopamine-induced inward current may be mediated by TAAR1.

Dopamine-induced inward current is blocked by EPPTB

To further characterize inward current evoked by dopamine, we performed experiments in sulpiride (1 μ M) in order to block D2 autoreceptor-mediated GIRK current. Superfusion with sulpiride began 15 min before adding dopamine (100 μ M), and I-V plots were constructed using voltage ramps as described above. Figure 10A shows subtracted (net) currents evoked by dopamine. The I-V plot after 2 min of dopamine superfusion showed no significant conductance (0.05 ± 0.07 nS, $n = 6$). However, mixed model analysis showed a significant difference between I-V plots at 10 min versus 2 min ($F_{(1,60)} = 27.365$, $P < 0.0001$). Conductance at 10 min (0.33 ± 0.13 nS, $n = 6$) was significantly greater than that recorded at 2 min ($t(5) = 3.400$, $P < 0.05$), with an E_{rev} estimated at -45 ± 24 mV. At 20 min, dopamine-induced conductance increased to 0.64 ± 0.33 nS, with an estimated E_{rev} of -75 ± 29 mV ($n = 4$). When comparing data at 10 versus 20 min, neither the conductance ($P = 0.432$) nor E_{rev} ($P = 0.460$) values differed significantly (Welch's t tests). Therefore, we combined data obtained at the 10 and 20 min time points and found that dopamine-evoked currents in sulpiride yielded an average slope conductance of 0.45 ± 0.25 nS and an E_{rev} of -57 ± 18 mV ($n = 10$).

The I-V plots in Fig. 10B show results of experiments in which slices were pretreated for 15 min with the TAAR1 antagonist EPPTB (3 μ M) plus sulpiride (1 μ M). Conductances for

dopamine-induced currents remained near zero when measured at 2 min (0.02 ± 0.08 nS, $n = 4$), 10 min (-0.03 ± 0.06 nS, $n = 4$) and 20 min (-0.06 ± 0.10 , $n = 4$). Because EPPTB completely blocked dopamine-induced inward current, this result supports the conclusion that dopamine-induced inward current may be mediated by activation of TAAR1.

DISCUSSION

Dopamine is well known to evoke a hyperpolarizing GIRK current in SNC dopamine neurons by activation of D2 autoreceptors (Lacey et al., 1987; Mercuri et al., 1997). However, our studies show that outward current generated by D2 receptor stimulation is opposed by an inward current that develops slowly and may be mediated by activation of TAAR1. The finding that DAT inhibitors block this inward current suggests that dopamine gains access to the intracellularly located TAAR1 by being transported into the neuron by the DAT. Our most surprising result, however, was that an AMPK blocking agent was able to reverse the ability of a DAT inhibitor to block the dopamine-induced inward current. To explain this result, we propose the hypothesis that a reduction in AMPK activity permits dopamine to gain entry into the cell through an alternate uptake site that differs from DAT, thus enabling activation of TAAR1. Figure 11 shows a schematic that illustrates our hypothesis for involvement of DAT, TAAR1, AMPK, and an alternate uptake site.

Dopamine is a TAAR1 agonist

TAAR1 is an intracellularly located G protein-coupled receptor that stimulates the production of cAMP (Lam et al., 2015). Moreover, TAAR1 is expressed in SNC dopamine neurons and can be activated by dopamine and its metabolites (Bunzow et al., 2001; Borowsky et al., 2001). Although SNC neurons contain dopamine, it is concentrated into synaptic vesicles where it presumably has limited access to TAAR1. Because our experiments showed that a DAT inhibitor can block the generation of TAAR1-dependent current, translocation of dopamine into the cell by DAT presumably enables contact with TAAR1. Furthermore, uptake of dopamine by DAT has been shown to effectively stimulate TAAR1 (Xie et al., 2007). Therefore, it is likely that DAT represents the primary mechanism by which dopamine gains intracellular access to TAAR1.

Alternate sites for dopamine uptake

Our finding that TAAR1 can be activated by dopamine in the presence of an AMPK inhibitor despite the presence of a DAT inhibitor suggests the existence of a modified or alternative uptake mechanism. It should be noted that several studies have shown that phosphorylation of DAT can alter its affinity for DAT inhibitors (Moritz et al., 2013; Challasivakanaka et al., 2017). It is possible that our use of AMPK modulators and/or prolonged dopamine superfusion altered DAT phosphorylation such that DAT inhibitors no longer effectively blocked dopamine uptake, which thereby permitted TAAR1 activation. However, dopamine might also gain access to the intracellular space by being taken up by low-affinity high-capacity transporters. One such transporter is the plasma membrane monoamine transporter (PMAT), which has been shown to translocate dopamine into the intracellular space (Miura et al., 2016). However, studies suggest that PMAT is not expressed by SNC dopamine neurons (Vialou et al., 2007). Another low-affinity high-

capacity uptake mechanism is the organic cation transporter 3 (OCT3), which is known to be expressed in the rat substantia nigra (Gasser et al., 2019). Although Mayer et al (2018) reported that midbrain dopamine neurons express OCT3 mRNA in mice, an immunohistological study by Cui et al (2009) found that OCT3 was expressed by astrocytes and non-dopamine neurons in the SNC but not by dopamine neurons. Although it is uncertain if dopamine neurons express OCT3, OCT3 has been shown to be insensitive to cocaine (Gasser, 2019; Mayer et al., 2018), which would agree with our finding that dopamine (in the presence of an AMPK inhibitor) can evoke an inward current despite the presence of a DAT inhibitor. If dopamine can be transported by OCT3 into dopamine neurons, our results would suggest that this transporter is tonically inhibited by AMPK. Unfortunately, we are not aware of any studies in the literature that have investigated an effect of AMPK on OCT3 function. However, AMPK is known to inhibit a variety of transporters and exchangers, including phosphate transporters, creatine transporters, and glutamate transporters (Sopjani et al., 2001; Shaw et al., 2017; Lang and Föller, 2014). Furthermore, one study reported that AMPK activation and OCT3 knockout had similar behavioral effects on social interaction behavior in male mice (Garbarino et al., 2018), which would be consistent with an inhibitory effect of AMPK on OCT3. Experiments are needed to test the hypothesis that AMPK exerts a tonic inhibition of a low-affinity, high-capacity uptake site such as OCT3.

Current generated by TAAR1

Many studies have shown that TAAR1 activation influences dopamine neuronal excitability, although whether these effects are excitatory or inhibitory remains controversial. Many papers have reported that trace amines exert an inhibitory influence on midbrain dopamine neurons (Leo et al., 2014; Revel et al., 2011), and spontaneous firing rates of midbrain dopamine neurons are increased in TAAR1 knockout mice (Lindemann et al., 2008). The inhibitory effect of trace amines has been shown to be caused by a reduction in a resting K^+ conductance (Bradaia et al., 2009). However, others have shown more complex effects of trace amines that are mediated indirectly by causing release of endogenous dopamine and/or alterations in D2 and GABAB receptor-mediated actions (Ledonne et al., 2011; Ledonne et al., 2010). In contrast, other studies have shown that TAAR1 activation increases the firing rate of midbrain dopamine neurons and increases dopamine output to target nuclei (Revel et al., 2012a; Revel et al., 2012b). Dave et al (2019) showed that TAAR1 activation evokes inward current by reducing a resting K^+ conductance in astrocytes, and this effect was mediated by the cAMP/PKA pathway. The cAMP/PKA pathway is commonly associated with neuronal excitation and facilitation of transmitter release (Shen and Surprenant, 1993; Cameron and Williams, 1993; Chavez-Noriega and Stevens, 1994), which would be consistent with our finding that TAAR1 activation evoked an excitatory current in SNC dopamine neurons. Our finding that this inward current developed over several minutes is also consistent with a G protein-coupled second messenger system. Although we have not identified the ionic channel activated by TAAR1, we estimated an E_{rev} of -57 mV, which could be consistent with a non-selective cation channel (NSCC). Because TAAR1 is positively linked to adenylyl cyclase, a cyclic nucleotide-gated channel is another possibility. Although dopamine neurons express the cyclic nucleotide-gated channel HCN, which generates H-current (Lacey and North, 1988; He et al., 2014), our studies showed that

EPPTB-sensitive inward current was present despite blocking H-current with ZD7288. Although a chloride-mediated conductance is another possibility, this is less likely because under our experimental conditions the expected E_{rev} for chloride (-83 mV) is more hyperpolarized than the E_{rev} estimated in our study. Although our data suggests that the dopamine-mediated inward current is mediated by a NSCC, further work is clearly needed to more fully identify and characterize the ion channel that mediates this current.

Regarding DAT uptake current

Because 2 Na^+ and 1 Cl^- are co-transported with dopamine during uptake by DAT, dopamine uptake is well known to generate an inward current (Mortensen and Amara, 2003; Ingram et al., 2002). However, we found that peak (2 min) dopamine current reversed direction at -113 mV, which is very close to that predicted by the Nernst equation for K^+ (-106 mV). Also, the I-V plot after two min of dopamine superfusion was essentially flat when D2 receptors were blocked by sulpiride. If there had been significant uptake current during peak dopamine-induced current, the E_{rev} should have been shifted to a more depolarized value, and an inward current should have been generated by dopamine in the presence of sulpiride. Although dopamine evoked an inward current after 10 min of superfusion, an uptake current should be present from the beginning of dopamine superfusion. One could argue that an absence of detectable uptake current could be due to our use of a high concentration of dopamine that encourages DAT internalization (Lohani et al., 2018). Also, we acknowledge that our use of gluconate in internal pipette solutions has been reported to shift the E_{rev} for uptake current (Ingram et al., 2002). However, our finding that dopamine-induced inward current could be blocked by a DAT inhibitor is consistent with ongoing dopamine uptake by DAT. But more to the point, our data suggests that the uptake of dopamine by DAT leads to activation of TAAR1, which subsequently generates inward current.

It should be noted that our results differ from those of Aversa et al (2018), who reported that a 10–15 min superfusion of 100 μM dopamine produced significant uptake current in SNC dopamine neurons in mouse brain slices. Unlike the dopamine-induced inward current in our studies, their inward current began within 1–2 min of starting superfusion, and it was generated by DAT because it was blocked by cocaine. In contrast, our studies showed that dopamine induced an inward current that was delayed in onset and was not blocked by a DAT inhibitor in the presence of an AMPK inhibitor. Although our studies and those of Aversa et al both used internal pipette solutions that contained gluconate and used tissue from young pups, species differences (mouse versus rat) could account for different results. Also, the studies by Aversa et al used low Ca^{2+} pipette solutions with little buffering capacity (0.1 mM Ca^{2+} and 0.75 mM EGTA) compared to our solutions (1 mM Ca^{2+} and 11 mM EGTA), which could significantly alter intracellular second messenger systems. It is possible that differing experimental conditions contribute to the different results of our study and those of Aversa et al.

Regarding the concentration of dopamine used in our studies

One could argue that our use of 100 μM dopamine is too high a concentration to be physiologically relevant. It is important to emphasize that we chose to use a high

concentration of dopamine in order to facilitate D2 autoreceptor desensitization. Although burst firing of dopamine neurons have been reported to generate synaptic concentrations of dopamine as high as 100 – 1000 μ M (Floresco et al., 2003; Goto et al., 2007), this high concentration of dopamine is undoubtedly transient. Nevertheless, high concentrations of dopamine do occur during times of intense synaptic activity, and our use of a high concentration of dopamine likely maximized our ability to characterize the TAAR1-dependent inward current. In addition to times of intense synaptic activity, animal studies have shown that supra-physiological levels of extracellular dopamine can be induced in the brain by amphetamine and cocaine (Stuber et al., 2019; Kalivas and Duffy, 1991), as does the use of levodopa in animal models of Parkinson's disease (Abercrombie et al., 1990). Thus, results of our findings might be relevant in clinical situations such as stimulant abuse and in use of levodopa in the treatment of Parkinson's disease.

Dopamine and D2 autoreceptor desensitization

In our previous study, we found that the rundown of quinpirole-induced current by AMPK activation required concurrent stimulation of D1-like receptors (Yang et al., 2019). Moreover, the slowing of quinpirole-induced current rundown by AMPK was mediated by the cAMP/PKA pathway, and the effect of AMPK was completely blocked by a D1-like antagonist. But unlike the D1 and D2 agonists used in our previous study, our focus on dopamine in the present study introduced an additional factor because dopamine is a substrate for DAT. Our data suggest that dopamine is transported into the neuron by DAT where it can activate a TAAR1-dependent NSCC. Moreover, the generation of inward current by TAAR1 activation contributed to the apparent rundown of dopamine-induced outward GIRK current as measured at -60 mV. Consequently, we conclude that the rundown of dopamine-induced current is a combination of D2 autoreceptor desensitization and concurrent generation of the TAAR1-dependent inward current. We conclude that AMPK influences dopamine-induced current rundown in two ways: by 1) slowing D2 autoreceptor desensitization, and 2) inhibiting the activation of TAAR1 by suppressing dopamine uptake by a low-affinity, high-capacity mechanism.

Functional considerations

Repeated doses of stimulant drugs have been shown to augment dopamine release in target nuclei and induce sensitization to the behavioral effects of these drugs (Banks and Gratton, 1995; Burger and Martin-Iverson, 1994). Results of the present study suggest that high concentrations of dopamine, such as might be caused by amphetamine or cocaine, can evoke TAAR1-dependent inward current. Although behavioral sensitization has been attributed to many factors, such as D2 autoreceptor desensitization and alterations in glutamate input to dopamine neurons (Madhavan et al., 2013; Argilli et al., 2008), it is possible that the excitatory influence of TAAR1-induced current could also play a role in increasing the excitability of dopamine neurons and augmenting dopamine release. By showing that AMPK slows D2 autoreceptor desensitization and has a tonic inhibitory influence on TAAR1-evoked current, our studies suggest that AMPK activators could dampen the excitability of dopamine neurons and might temper the development of behavioral sensitization to stimulant drugs.

In summary, our studies show that dopamine can evoke an inward current that is blocked by a TAAR1 antagonist. TAAR1 activation is made possible by translocating dopamine into the neuron by DAT. Our results also show that inhibitors of AMPK permit the activation of TAAR1 despite the presence of DAT blockers. Our results are consistent with a model in which a low-affinity, high-capacity uptake site is tonically inhibited by AMPK. Consequently, AMPK inhibitors would permit TAAR1 activation by disinhibiting the non-DAT uptake site. Taken together with results from our earlier studies, our findings suggest that AMPK can reduce dopamine neuronal excitability by inhibiting TAAR1 activation and by reducing D2 autoreceptor desensitization.

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Abbreviations

AMPK	AMP-activated protein kinase
DAT	dopamine transporter
E_{rev}	reversal potential
GIRK	G protein-coupled inwardly rectifying K ⁺ channel
I-V	current-voltage
NSCC	non-selective cation channel
OCT3	organic cation transporter 3
PMAT	plasma membrane monoamine transporter
SNC	substantia nigra zona compacta
TAAR1	trace amine-associated receptor 1
VTa	ventral tegmental area

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Highlights

- Dopamine evokes a slowly developing inward current in SNC neurons
- This inward current is blocked by DAT inhibitors
- AMPK inhibitors permit the emergence of inward current despite DAT inhibition
- Dopamine-induced inward current is blocked by a TAAR1 antagonist
- We conclude that dopamine evokes a TAAR1 current modulated by AMPK

Fig. 1. Schematic illustrating the pathways by which AMPK slows the desensitization of D2 autoreceptors.

Our previous work showed that slowing of D2 autoreceptor desensitization by AMPK requires activation of PKA by D1 receptor stimulation (Yang et al., 2019).

Fig. 2. SCH39166 reduces but does not completely block the ability of A769662 to slow rundown of dopamine-induced current.

Currents were recorded at -60 mV with pipettes that contained either normal internal solution (control) or A769662 (10 μ M). Slices were superfused continuously with dopamine (100 μ M), and sulpiride (1 μ M) was added to the superfusate during the last 5 min. **A**) Dopamine-induced outward current runs down rapidly under the control condition, but A769662 markedly slowed the rundown of dopamine-induced current. The D1-like antagonist SCH39166 (1 μ M), which was superfused continuously beginning 10 min before dopamine, caused only a partial block of the effect of A769662 on rundown of dopamine-

induced current. Each current trace was recorded from a different neuron. B) Summary graph showing that the slowing of dopamine-induced current rundown by A769662 was significantly reduced by SCH39166 (*). However, the rundown of dopamine-induced current with A769662 plus SCH39166 remained significantly slower compared to dopamine with SCH39166 but without A769662 (#). C) The increase in sulpiride-sensitive current by A769662 was not significantly reduced by SCH39166. Current-decay plots were analyzed with a mixed model followed by Sidak pairwise comparison tests, whereas sulpiride-sensitive currents were analyzed with Welch's *t* test: * or #, $P < 0.05$; ** or ##, $P < 0.01$; *** or ###, $P < 0.001$.

Fig. 3. The AMPK blocker dorsomorphin prevents the effects of GBR12935 and A769662 on the rundown of dopamine-induced current.

A) Summary graph showing that the DAT inhibitor GBR12935 (1 μ M in superfusate) plus A769662 (10 μ M in pipette) caused significant slowing of current rundown evoked by dopamine (100 μ M). Dorsomorphin (30 μ M) completely blocked the combined effects of A769662 plus GBR12935 (*). B) GBR12935 significantly increased sulpiride-sensitive current in the presence of A769662. The addition of dorsomorphin significantly reduced the sulpiride-sensitive current recorded in A769662 and GBR12935. SCH39166 (1 μ M) was present in all recordings to block possible activation of D1-like receptors. Current-decay plots were analyzed with a mixed model followed by Sidak pairwise comparison tests, whereas sulpiride-sensitive currents were analyzed with Welch's t tests: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 4. AMPK blocker prevents the ability of GBR12935 to slow rundown of dopamine-induced current.

A) Outward current evoked by dopamine (100 μ M) decays to zero despite continuous superfusion. B) The DAT inhibitor GBR12935 (1 μ M) slows the rundown of dopamine-induced current and reveals significant residual current that is blocked by sulpiride (1 μ M). C) The AMPK blocking agent dorsomorphin (30 μ M) prevents the ability of GBR12935 to slow the rundown of dopamine-induced current. Sulpiride (1 μ M) was superfused at the end of recordings to test for residual current mediated by D2 autoreceptors. Each recording was from a different neuron with voltage clamped at -60 mV.

Fig. 5. Dorsomorphin and STO609 block effects of GBR12935 on dopamine-induced currents. A) Summary graph showing that the effect of GBR12935 on rundown of dopamine-induced current is prevented by pretreatment with either dorsomorphin or STO609. B) Dorsomorphin and STO609 completely block sulpiride-sensitive current recorded with GBR12935. Current-decay plots were analyzed with a mixed model followed by Sidak pairwise comparison tests, whereas sulpiride-sensitive currents were analyzed with Welch's *t* tests: **, $P < 0.01$, ***, $P < 0.001$.

Fig. 6. Dorsomorphin prevents the ability of cocaine to slow rundown of dopamine-induced current.

A) Summary graph showing that the cocaine (10 μ M)-induced slowing of dopamine-induced current rundown (*) is antagonized by pretreatment with dorsomorphin (#). B)

Dorsomorphin completely blocks sulpiride-sensitive current recorded with cocaine. Current-decay plots were analyzed with a mixed model followed by Sidak pairwise comparison tests, whereas sulpiride-sensitive currents were analyzed with Welch's *t* tests: * or #, $P < 0.05$; ** or ##, $P < 0.01$, *** or ###, $P < 0.001$.

Fig. 7. Dorsomorphin fails to alter current evoked by quinpirole plus SKF38393.

Slices were superfused continuously with quinpirole (30 μ M) and SKF38393 (10 μ M) for 25 min with and without dorsomorphin. A) Rundown of current evoked by quinpirole plus SKF38393 is not altered by dorsomorphin pretreatment. B) Dorsomorphin does not alter sulpiride-sensitive current evoked by quinpirole plus SKF38393.

Fig. 8. Current-voltage (I-V) plots show that GBR12935 blocks inward current evoked by dopamine.

A) Raw current traces recorded before (control) and at various times after beginning dopamine superfusion. Voltage ramps were begun after holding potentials at -120 mV for 0.5 sec. Peak dopamine current, which was recorded 2 min after beginning superfusion, shows an E_{rev} near that expected for K^+ , whereas currents recorded at 10 and 20 min show inward shifts. B) Summary graph showing the voltage-dependence of net (subtracted) currents evoked by dopamine. Currents recorded at 10 and 20 min show significant

depolarized shifts in E_{rev} . C) GBR12935 prevents the depolarizing shift in E_{rev} induced by dopamine.

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Fig. 9. Dorsomorphin and TAAR1 antagonist EPPTB modify the effect of GBR12935 on dopamine-induced currents.

A) Dopamine induces inward shifts in I-V plots when slices are superfused with dorsomorphin in the presence of GBR12935. B) Addition of EPPTB (3 μ M) to the superfusate prevents the development of dopamine-induced inward current in the presence of dorsomorphin and GBR12935.

Fig. 10. EPPTB blocks dopamine-induced inward current.

Slices were superfused continuously with sulpiride (1 μ M) to block D2 autoreceptor-mediated K^+ current. A) Dopamine induces progressively greater inward shifts in I-V plots 10 and 20 min after starting dopamine superfusion. Dopamine induces no significant current 2 min after starting superfusion. B) The TAAR1 antagonist EPPTB (3 μ M) completely blocked the development of dopamine-induced inward current.

Fig. 11. Schematic showing hypothesized interactions between DAT, TAAR1, and AMPK. Dopamine normally is taken up into cells via DAT where it can stimulate TAAR1, which secondarily activates a non-selective cation channel (NSCC). DAT blocking agents prevent TAAR1 activation by reducing the uptake of dopamine. However, dopamine may enter the neuron through an alternate uptake pathway, which we hypothesize may be under tonic inhibition by AMPK. Therefore, AMPK blocking agents may relieve the alternate uptake pathway from inhibition, which allows dopamine to gain intracellular access, activate TAAR1, and activate NSCC despite continued block of DAT. The net effect of TAAR1 activation is to evoke an inward current that opposes the outward current evoked by D2 autoreceptor activation of GIRK.