MODULATION OF AMPA CURRENTS BY D₁-LIKE BUT NOT D₂-LIKE RECEPTORS IN SPINAL MOTONEURONS

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Abstract—Dopamine can modulate and excite spinal locomotor networks, affect afferent transmission and increase motoneuronal excitability. One of the mechanisms whereby dopamine increases motoneuronal excitability is to potentiate AMPA channel-mediated glutamatergic transmission onto motoneurons. However, it is not known which dopaminergic receptor subtypes or the intracellular mechanisms contribute to these effects. In this study, we used whole-cell patch clamp techniques to record chemically evoked AMPA currents in neonatal mouse motoneurons. Bath application of D₁-like receptor agonist (SKF 39383) increased the AMPA current amplitude and prolonged the decay time constant. In the presence of D₁ receptor antagonist LE300, the effects of DA on AMPA currents were blocked. In contrast, bath-application of the D₂-like receptor agonist quinpirole did not modulate AMPA currents. In the presence of D₂ receptor antagonist L-741626, dopaminergic modulation of AMPA currents was unaffected. These results suggest that augmentation of AMPA transmission by dopamine is accomplished by D₁ receptor-based mechanisms. This short-term modulation does not appear to involve cycling of AMPA receptor into the membrane, since blocking insertion with botulinum toxin C did not affect the augmentation of AMPA currents after activating D₁ receptors. On the other hand, blocking protein kinase A (PKA) with H-89 completely abolished the effects of D₁ agonists. In addition, we used cell-attached single channel recording to demonstrate that stimulating D₁ receptors increased individual AMPA channel open probability and open duration. Our data demonstrate that dopamine increases the efficacy of glutamatergic transmission onto motoneurons by increasing AMPA conductances via a D₁, PKA-based signaling system. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: dopamine, AMPA channel, PKA, motoneuron, spinal cord.

Dopamine (DA) is known to have multiple roles in modulating spinal sensorimotor function. For example, DA can modulate and excite locomotor networks within the spinal cord (Kiehn and Kjaerulf, 1996; Jiang et al., 1998; Whelan et al., 2000; Barriere et al., 2004; Madriga et al., 2004; Christie and Whelan, 2005), affect afferent transmission and increase motoneuronal excitability (Han et al., 2007). Our previous work (Han et al., 2007) demonstrated that DA has multiple effects on motoneuronal intrinsic properties. DA depolarizes motoneurons, decreases both the SKca and the I₄ conductivity, and increases the input resistance. The net result is that the gain or the input–output relationship of motoneurons is dramatically increased when DA is present. DA also acts to boost glutamatergic transmission onto motoneurons by increasing AMPA channel-mediated currents. However, it is not known which receptor subtypes contribute to these effects on synaptic transmission.

Dopaminergic receptors are a large family of G-protein-coupled receptors with diverse functions (Emilien et al., 1999). Five different classes of DA receptors have been cloned within the CNS. These five subgroups can be divided into two general families of receptors, namely the D₁-like and D₂-like receptor classes based on structural similarities, and the fact that each family has excitatory and inhibitory actions respectively on neurons. The D₁-like family includes D₁ and D₅ receptor subtypes, while the D₂-like family includes D₂, D₃, and D₄ receptor subtypes. Genomically, the D₁-like family has no introns (Sunahara et al., 1990; Zhou et al., 1992), whereas the D₂-like family contains introns within the coding region (Araki et al., 1992; Deinard and Kidd, 1998). Furthermore, due to different splicing, D₂ subtypes can be a short form (D₂s) or a long form (D₂l) (Giros et al., 1989). D₁-like receptors are G-protein coupled and activate adenylyl cyclase leading to an increase in cAMP. On the other hand, D₂-like receptors inhibit adenylyl cyclase and decrease cAMP (Greenberg, 2001). DA receptors are widely distributed in the CNS, as well as cardiovascular and endocrine systems. The mRNA for D₁–D₅ receptor subtypes is expressed in the spinal cord (Zhu et al., 2007). The most abundant subtypes in ventral horn are D₁ and D₃ subtypes, with D₄ and D₅ present less abundantly (Zhu et al., 2007). In addition, dopaminergic fibers project from the diencephalon (area 11) and are found in all Rexed’s laminae (Skagerberg et al., 1982; Qu et al., 2006). Lesions of area 11 in mice lead to a reduction in the release of DA within the lumbar spinal cord of mice (Zhao et al., 2007).

Previous work in other CNS regions has identified several DA actions on synaptic transmission that are relevant for DAergic modulation of spinal cord circuits. In the striatum and nucleus accumbens of mammals, DA, acting though D₁ receptors, can increase NMDA and AMPA conductances (Greengard, 2001). The signaling mechanism is a G-protein-coupled system that increases the activity of cAMP and protein kinase A (PKA) that ultimately leads to phosphorylation of AMPA and NMDA ion channels. Our previous work shows that DA increases AMPA transmis-
sion (Han et al., 2007), which supports previous findings obtained using cultured chick motoneurons (Smith et al., 1995). Here we provide evidence showing that DA acts to increase AMPA synaptic transmission by D<sub>1</sub>-like receptor-mediated mechanisms. A portion of these results have been published in abstract form (Han and Whelan, 2007).

**EXPERIMENTAL PROCEDURES**

Experiments were performed on Swiss Webster mice (Charles River Laboratories, St-Constant, Quebec, Canada) 3–8 days old (n=78). The animals were anesthetized by hypothermia (age ≤ P4) or by halothane (>P4) using procedures conforming to the Canadian Council on Animal Care guidelines and approved by the University of Calgary Animal Care Committee.

**Drugs and chemicals**

DA and AMPA were purchased from Sigma-Aldrich (St. Louis, MO, USA). SKF 38393, quinpirole, LE300, H-89 and L741626 were obtained from Tocris Bioscience (Ellisville, MO, USA). Botulinum toxin C (botox C) stock solution was a gift from the laboratory of Dr. J. Bains. DA (50 μM) was dissolved in distilled water and stock solutions were prepared fresh prior to each experiment. Other pharmacological reagents including SKF 38393, quinpirole, L741626 and LE300 were prepared in DMSO stock solution and diluted to the final solution.

**Tissue preparation**

The procedure for tissue preparation has been documented in detail in our previous publication (Han et al., 2007). Briefly, the spinal cord was dissected free in ice-cold, oxygenated sucrose–ACSF solution (concentrations in mM: 128 NaCl, 4 KCl, 1.5 CaCl<sub>2</sub>, 1 MgSO<sub>4</sub>, 0.5 Na<sub>2</sub>HPO<sub>4</sub>, 21 NaHCO<sub>3</sub>, 30 glucose and 1 μM TTX). Cs<sup>+</sup>-internal solution was used to limit K<sup>+</sup> currents, minimize leak conductances, and improve space clamp. The internal pipette solution comprised (in mM): 119 NaCl, 1.9 KCl, 1 CaCl<sub>2</sub>, 10 MgSO<sub>4</sub>, 1.2 Na<sub>2</sub>HPO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 10 glucose, and equilibrated for at least 45 min before being placed into the recording chamber for IR-DIC visually guided patch clamp recordings.

**Patch clamp recordings**

The lumbar spinal cord slice was placed into the recording chamber and superfused with oxygenated ACSF solution. The external solution contained (in mM): 128 NaCl, 4 KCl, 1.5 CaCl<sub>2</sub>, 1 MgSO<sub>4</sub>, 0.5 Na<sub>2</sub>HPO<sub>4</sub>, 21 NaHCO<sub>3</sub>, 30 glucose and 1 μM TTX. Cs<sup>+</sup>-internal solution was used to limit K<sup>+</sup> currents, minimize leak conductances, and improve space clamp. The internal pipette solution comprised (in mM): 150 CsCl, 1 EGTA, 10 Hepes, 0.1 CaCl<sub>2</sub>, 4.6 MgCl<sub>2</sub>, 2 ATP, and 0.5 GTP. AMPA (100 μM) was puffed onto the targeted cells using a Clampex protocol to trigger the Picospritzer. The puffer pipette contained AMPA, along with the ACSF used in the experiments. The flow rate was set at an approximate 2 ml/min. Electrodes were pulled from borosilicate glass on a P87 Flaming/Brown puller (Sutter Instrument, Novato, CA, USA) and had resistances in the range of 3–5 MΩ. The liquid junction potential between internal and external solutions was calculated using pClamp software to be 1.4 mV and corrected. Motoneurons were visually identified by their characteristic large soma diameter and location using differential interference contrast optics and infrared light transmission. In our previous publication, we used FG injection to verify that all the cells we recorded were motoneurons, so we are confident in our method of visual identification. The data were low pass filtered (10 kHz) and digitized (sampling rate: 20 kHz) for offline analyses (Digidata 1322A, Clampex and Clampfit 8 and 10, Molecular Devices, Union City, CA, USA). The puff electrode was advanced to an area just above the target cell. The target cell was then whole cell patched. A triggering protocol was set up in Clampex to trigger the Picospritzer and record the resultant current (Duration of puff: 500 ms; one puff every minute for duration of the protocol; data sweep recorded for 30 s). At the end of each trace, a 10 mV square voltage step was given to monitor the input resistance. For whole cell recordings, the pipette solution contained (in mM): CsCl, 140; tetraethylammonium chloride, 10; 4-aminopyridine 0.2; CaCl<sub>2</sub>, 1; Hepes 5, and 1 μM TTX, pH 7.3 with CsOH (Xu et al., 1999). This solution was chosen to eliminate several voltage-gated conductances. AMPA stock solution was diluted into the pipette solution to reach a final concentration of 100 nM. In our preliminary test, we excluded AMPA from internal solution and no single channel events were observed. The cell-attached patches were held at 30 mV (the transmembrane potential was estimated to be ~60–80 mV). Continuous recordings were for no less than 60 s under control and SKF 38393 conditions. The data-recording segment under SKF 38393 was regarded as valid only after superimposing SKF38393 for at least 3 min (time corresponding to the significant effects in whole cell experiments).

**Data analysis**

Data analyses were performed using Clampfit and Origin 5.0 (Microcal, Northhampton, MA, USA). For whole cell recordings, the peak amplitude was calculated and a first order exponential function was applied to the decay phase of the current to determine the time constant (Shanfullina et al., 2004). Data were normalized to the peak amplitude of the first evoked current. A repeated measures ANOVA was performed. If significant effects were observed, a post hoc Tukey test (all data points compared with the first trace) was performed. For cell-attached recordings, a minimum duration of 30 s was analyzed to calculate event detection using Clampfit-10 single-channel analysis functions. The amplitude histogram was plotted and fitted with a gaussian function to calculate the average single channel amplitude. The dwell time was plotted and fitted with a weighted exponential function (based on Poisson models) to calculate the average channel open duration (dwell time). The open probability was calculated as the total time duration that the recorded channel stays open divided by the total recording time. The average amplitude, open dwell time and open probability were compared before and after applying SKF 38393 using a paired Student’s t-test.

**RESULTS**

**D<sub>1</sub>-like effects**

During each experiment, we recorded the evoked current following AMPA puff stimulation every minute for a total of 10 min. We observed no significant desensitization or facilitation of the evoked currents during our control trials (intertrial interval 1 min; Fig. 1C, D). In our previous study, we found that DA increased motoneuronal input resistance when K-glucerate internal solution was applied, the changes of input resistance appear to be predominantly contributed by K<sub>leak</sub> currents. We did not observe significant changes of input resistance contributed by DA when we used Cs<sup>+</sup>-internal solution (Han et al., 2007).

The D<sub>1</sub> receptor agonist SKF 38393 (20 μM) was bath-applied from the 10th minute for 5 min. We observed a significant increase in the amplitude of the evoked AMPA currents between the 14th min and 16th min (32.2 ± 10.3%....
by changes of input resistance, since it did not significantly change during the recording (133.3 ± 5.3 MΩ before bath application of SKF 38393, 136.8 ± 5.6 MΩ during bath application of SKF 38393, and 136.1 ± 4.8 MΩ after washout (P > 0.05, repeated measures ANOVA)).

We next tested whether the DA-mediated increase in AMPA currents was mediated through a D₁-receptor-like pathway. Preceding DA application, we superfused the slice with the D₁ receptor antagonist LE300 (10 nM) to pre-block the D₁ receptors. LE300 by itself did not increase or decrease the AMPA current amplitude or time constant, suggesting that there is little constitutive activity of D₁ receptors in the test cells. In the continuous presence of LE 300 (10 nM), DA did not increase the AMPA current amplitude (the maximal change was −23.1 ± 12.9%, P > 0.05, N = 6, Fig. 2A) or prolong the decay time constant (the maximal change was 22.4 ± 13.2%, P > 0.05, N = 6, Fig. 2B). The input resistance remained stable during the course. The average input resistance was 139.9 ± 8.6 MΩ in the beginning, 139.2 ± 6.9 MΩ in the presence LE300 alone, and 141.4 ± 4.7 MΩ in the presence of both LE300 and DA (P > 0.05). Overall these data suggest that D₁-like receptors are mediating the effect of DA on AMPA channels.

Fig. 1. The D₁ receptor agonist SKF 38393 (20 μM) increased the amplitude and time constant of the evoked AMPA currents. (A) An example of puff-evoked whole cell AMPA currents. (B) Normalized traces from A show the change in decay time (expressed as time constant). Control (black trace), SKF (dark gray trace) and Wash (light gray trace). (C) SKF 38393 (20 μM) increased the amplitude of the evoked AMPA currents. (D) SKF 38393 (20 μM) also increased the decay time constant. * Indicates a significant difference (P < 0.05) compared with the first normalized trace, N = 7.

Fig. 2. D₁ antagonist blocks DA modulation of AMPA currents. In the presence of D₁ antagonist LE300 (10 nM), DA did not increase AMPA current amplitude (A) or normalized decay time constant (B). N = 7.
D2-like receptors

We next turned our attention to D2-like receptors. However, in contrast to the D1-mediated effects, bath application of the D2 agonist quinpirole (20 μM) did not significantly change AMPA currents, even when we extended (10 min) the application of quinpirole in the bath solution (Fig. 3A, B). During the bath-application of quinpirole, the largest amplitude decrease was no more than 5.1 ± 4.7% (P > 0.05). The largest decrease in time constant was no more than 17.4 ± 15.8% (P > 0.05). During the course, the input resistance remained stable (beginning: 135.1 ± 8.4 MΩ, quinpirole: 130.1 ± 7.1 MΩ, P > 0.05). Only large concentrations of the D2-like receptor agonist quinpirole (50 μM) reduced the AMPA current amplitude (the maximum decrease was 20.3 ± 3.1%, P < 0.05), and shortened the decay time constant (maximum decrease was 14.7 ± 3.5%, N = 5). However, these high concentrations are a concern since nonspecific interactions may occur that may not be attributed to D2 receptors.

As an additional control we next bath applied DA to determine whether D2 antagonists could unmask an inhibitory effect of the D2 receptors. Administration of the D2 receptor antagonist L-741626 (20 nM) did not significantly change the AMPA amplitude or decay time constant (Fig. 4A, B, data points from 5th to 10th min), suggesting there is no observable constitutive activation of D2 receptors on motoneurons. With D2 receptor pre-blocked by L-741626, DA remained capable of increasing the amplitude and decay time constant of AMPA currents (Fig. 4A, B, after 10th min). The amplitude increment amounted to 47.5 ± 15.4% (P < 0.01, N = 4) at the 14th min, 49.3 ± 17.8% (P < 0.01, N = 4) at the 15th min and 41.9 ± 21.8% at the 16th min (P < 0.05, N = 4). The maximal decay time constant increased to 27.5 ± 9.5% at the 19th min (Fig. 4B, N = 4). During these experiments, the input resistance remained unchanged (beginning: 139.1 ± 10.9 MΩ, L-74162: 137.6 ± 9.7 MΩ, L-74162 + DA: 135.6 ± 11.0 MΩ, P > 0.05). Overall our data show that activation of D1 receptors modulates motoneuronal AMPA currents. D1-like receptor activation mimics DA potentiation of the AMPA currents in motoneurons, whereas we could find no evidence that D2-like receptors participate in this process.

Mechanisms underlying D1 modulation of AMPA currents

The modulation of AMPA current could be caused by insertion of additional AMPA receptors into the membrane (Malenka, 2003; Sun et al., 2008), as well as by a direct phosphorylation of AMPA channel receptors (Wolf et al.,...
To test the first possibility, we included botox C (5 μg/ml) into our intracellular pipette solution to block AMPA channels from mobilizing between membrane and internal reserves (Gordon and Bains, 2005). D₁-like receptor agonist SKF 38393 (20 μM) was still able to increase the AMPA currents even in the presence of botox C. The amplitude was increased by 33.4 ± 8.1% (P < 0.01) at the 8th min, 34.6 ± 6.9% (P < 0.01) at the 9th min, 37.9 ± 8.9% (P < 0.01) at the 10th min and 39.1 ± 8.5% (P < 0.01) at the 11th min (N = 8, Fig. 5A). The decay time constant was also increased by 35.1 ± 14.9% (P < 0.05) at the 7th min, 33.3 ± 11.1% (P < 0.05) at the 8th min and 32.9 ± 10.2% (P < 0.05) at the 9th min (N = 8, Fig. 5B). This increase was comparable to the effects of SKF 38393 in the absence of botox C, suggesting that the increases in AMPA current amplitude, at least during the short duration of 5 min bath-application of D₁ receptor agonist, were not due to an increase in the number of membrane bound AMPA receptors.

Next, we included the PKA blocker H-89 (20 μM) into the pipette solution to test the role of phosphorylation. Blocking phosphorylation results in a spontaneous rundown of AMPA current amplitude similar to the situation where intracellular ATP is insufficient (Lin et al., 2003), suggesting a constitutive phosphorylation is necessary for stabilizing AMPA currents (Fig. 6A). The maximal rundown is −35 ± 3.4%, (P < 0.01 compared with the first normalized trace, N = 6). However, blocking phosphorylation did not result in significant changes in AMPA current time constant (Fig. 6B). The largest change in time constant was 2.6 ± 6.9% (P > 0.05, N = 6). Under this condition, D₁ receptor agonist SKF 38393 (20 μM) did not change the time course of rundown. For each data point, there is no significant difference compared with the corresponding data point in the control condition (square and round symbols; Fig. 6A). SKF 38393 did not change the time constant either (square and round symbols in Fig. 6B). This result suggests that DA activation of D₁-like receptors involves activation of the cyclic AMP/PKA (cAMP/PKA) signaling cascade.

If the increment in AMPA current depends on phosphorylation but not on additional membrane insertion, a likely target for the D₁-mediated effects is on individual AMPA channel properties. We tested this possibility using cell-attached configuration to obtain single channel recordings. We established single channel somatic recording patches in close proximity to dendrites. Clear closed-open bistable states can be discerned in the raw traces (Fig. 7A). To test D₁ receptor activation, we first superfused the D₁ agonist SKF 38393 (20 μM), and subsequently gave another stimulation by superfusing a mixture of SKF 38393 and H-89.
(20 μM) and cAMPS-Rp (50 μM), a membrane permeable PKA inhibitor (Fig. 7A). Although the D₁ agonist SKF 38393 (20 μM) increased whole cell AMPA current amplitude (Fig. 1), it did not change the unitary AMPA current amplitude (Fig. 7B and C). The single channel amplitude (cell attached V₉₀=30 mV) was 2.0±0.3 pA before and 2.1±0.4 pA after applying SKF 38393. In the presence of both SKF 38393 and cAMPS-Rp, the average unit amplitude was 1.9±0.2 pA (N=10, P>0.05, repeated-measures ANOVA). Instead, D₁-like receptor activation increased the average dwell times on opening configuration (open time) from 64.6±10.9 ms to 87.5±15.4 ms (P<0.05, repeated-ANOVA with post hoc test, N=10, Fig. 7D). In the presence of

![Figure 7](https://example.com/figure7.png)

**Fig. 7.** D₁-receptor agonist SKF 38393 (20 μM) increased AMPA channel open probability and open duration. (A) An example recording from cell-attached configuration showing the opening (O) and closing (C) status in the absence (control), or presence of SKF 38393 (abbreviated as SKF), and in the presence of both SKF 38393 and cAMPS-Rp (SKF plus cAMPS-Rp). (B) Event count collected from the same patch as shown in A. In the amplitude histograms (left column), the events of open status were plotted and fitted with a gaussian function. Notice the number of open events was increased in SKF 38393, and decreased toward the control levels in the presence of SKF+cAMPS-Rp, but the peak amplitude remained the same. The bin width was 0.5 pA. In the open dwell time histogram, the open status events were plotted and fitted with weighted exponential function to calculate the average dwell time in open status (open time). The bin width is 1 ms. (C–E) Summary of the statistical results of amplitude, open time and open probability respectively, in the absence (control, white bar), or in the presence (black bar) of SKF 38393, and in the presence of both SKF and cAMPS-Rp (N=10). * Indicates P<0.05 compared with control (repeated-measures ANOVA with Tukey post hoc test).
open time was 62.5 ± 8.7 ms, which is significantly different from the effect of SKF 38393 alone (P < 0.01, N = 10) but not different from control values (Fig. 7D). The channel open probability was calculated as the ratio of cumulative duration on open configuration over total recording duration. The open probability was 14.5 ± 5.7% at control and was increased to 23.8 ± 6.2% after applying SKF 38393 (N = 10, P < 0.05, Fig. 7E). In the presence of both SKF 38393 and cAMPS-Rp, the open probability was 8.8 ± 3.0%, which was significantly different from stimulation by SKF 38393 alone (P < 0.01) but not different from that of control (P > 0.05, N = 10, Fig. 7E). Thus, activating D₁-like receptors increases open probability and open durations within individual AMPA channels. PKA is likely to participate in the signaling pathway from D₁-like receptors to AMPA channels since stimulating D₁ receptors did not affect AMPA channel open probability or open time in the presence of PKA inhibitors.

We also recorded the single channel events using the D₂ agonist quinpirole (20 μM). Addition of quinpirole produced no significant modulation of the unit current amplitude, open probability or the open time of individual AMPA channels. The AMPA channel unit current amplitude was 2.3 ± 0.2 pA before and 2.4 ± 0.5 pA after applying quinpirole (P > 0.05, N = 9, paired t-test). The open time was 78.1 ± 18.7 ms before and 86.3 ± 16.8 ms after applying quinpirole (P > 0.05, N = 9). The open probability was 15.6 ± 3.8% before and 17.5 ± 6.0% after applying quinpirole (P > 0.05, N = 9). Taken together, activating D₁-like receptors significantly modulated the probability of individual AMPA channels, whereas activating D₂-like receptors produced no significant modulatory effects.

**DISCUSSION**

The effect of DA on striatal and ventral tegmental synaptic transmission has been extensively examined, in part driven by the need to elucidate the pathophysiology of Parkinsonism and drug addiction (Greengard, 2001). The effects of DA on motor function are usually explained through indirect projections to the spinal cord. However, it is clear that DA also contributes to modulation of spinal cord circuits, although there is a considerable gap in our knowledge of its actions compared with 5-HT and noradrenaline (Rekling et al., 2000). Our previous study showed that DA directly modulates both interneurons and motoneurons within spinal cord circuits likely contributing to the control of locomotion (Han et al., 2007). Specifically, DA directly modulates ion channels and synaptic transmission on spinal motoneurons, the final common pathway for motor control. Evidence from our previous work suggests that postsynaptic AMPA channels are one of the targets for dopaminergic modulation (Han et al., 2007). Our current work examines the dopaminergic receptor(s) involved and their downstream signaling pathways. A caveat of this study is that we focus on the families of D₁ and D₂-like receptors. That said, we demonstrate that D₁-like receptors, but not D₂-like receptors, are likely responsible for mediating DA’s modulation of AMPA currents. Within the time frame of several minutes, it appears to be a PKA-dependent mechanism, consistent with other reports of D₁ postsynaptic effects within the CNS (Price et al., 1999; Wolf et al., 2003). Our data suggest that over short periods, activation of D₁-like receptors increases the open probability and open duration of AMPA channels. Our data provide one mechanism whereby activation of D₁ receptors can increase the excitability of spinal motor networks in rats (Seth et al., 1993; Barriere et al., 2004) and mice (Madriaga et al., 2004).

In combination with our previous work demonstrating that DA enhances motoneuronal sEPSCs and mEPSCs and enhances puff-evoked AMPA currents (Han et al., 2007), our work strongly suggests that DA’s modulation of AMPA channels through D₁-like receptors is an important mechanism contributing to motoneuron excitability. Similar mechanisms have been reported in prefrontal cortex where DA or D₁-receptor agonist enhances glutamate currents whereas D₂-agonist fails to do so (Gonzalez-Islas and Hablitz, 2003). However, our observation that D₁ receptors act to increase AMPA currents does not take into account presynaptic changes in glutamate release. For example, DA depresses, rather than enhances, excitatory synaptic transmission onto rat subicular neurons via postsynaptic D₁-like DA receptors (Behr et al., 2000). We cannot rule out that presynaptic effects are occurring in premotor interneurons or afferent terminals in the spinal cord, but the primary postsynaptic effect of DA acting on D₁ receptors is to boost AMPA transmission in other brain regions and our work confirms this for the mammalian spinal cord.

Although we failed to detect any specific effect of D₂ receptors on modulating AMPA channels on motoneurons, D₂ receptors are predominantly expressed in spinal motoneurons. In fact, RT-PCR detected mRNA copies that were higher than D₁ (Zhu et al., 2007). The absence of a direct role of D₂ receptors in modulating AMPA receptor does not preclude their potential participation in modulating neuronal excitability. For example, D₂ receptor indirectly attenuates local excitatory synaptic transmission in the adult PFC by increasing local GABAergic synaptic transmission (Tseng and O’Donnell, 2007). D₂ receptor activation reduced NSF–GluR2 interaction resulting in a decrease of AMPA receptor membrane expression (Zou et al., 2005). To reveal this effect seems to require a long time duration in cell cultures so it likely evades our detection in our acute experiment. Also D₂ actions may have an effect on intrinsic properties of neurons in the ventral horn, which were not examined here. A final caveat is the possibility that the PKA consensus site is fully dephosphorylated during application of D₂ agonists. Our data cannot fully exclude this possibility. A comprehensive understanding of how DA modulates spinal motoneuronal excitability needs to take into account presynaptic effects on premotor interneurons and afferent terminals, as well as time-dependent plastic effects (Nicola and Malenka, 1997; Barriere et al., 2008).

Insertion of AMPA channels into silent synapses within the time frame of 30–60 min often leads to long-term
plasticity (Malenka, 2003). Over long time frames, application of D1-agonist increases the AMPA surface expression irreversibly in nucleus accumbens neurons cocultured with prefrontal cortex neurons (Sun et al., 2008). In our study we controlled for this possibility, but as expected did not observe that AMPA channel insertion plays a critical role in the short-term AMPA current enhancement. Instead our data show that direct phosphorylation by PKA of AMPA channels is a possible mechanism, since the enhancement of AMPA currents by D1 agonists cannot be induced by blocking intracellular PKA pathways. Blocking PKA results in a rundown phenomenon. Although a linear rundown in AMPA current was previously reported in striatum spiny medium neurons (Lin et al., 2003), the rundown induced by blocking PKA activity in our work is more rapid and non-linear. Adding D1-receptor agonist failed to reverse or slow the rundown. This is in agreement with published work suggesting that PKA phosphorylates the GluR1 Ser845 site in nucleus accumbens (Chao et al., 2002), where H-89 could pre-block the phosphorylation.

Activating D1-like receptors increases individual AMPA channel open probability and open duration. This effect is likely induced by mobilizing PKA activity because reconstituted PKA directly phosphorylates GluR1 and increases single channel open probability while keeping the single channel conductance unchanged (Banke et al., 2000). Since synaptic AMPA channels are inaccessible, our samplings of single AMPA channel currents were performed on the soma surface in proximity to the dendrites. Although we do not know how much the synaptic and extrasynaptic AMPA channel properties differ from each other functionally, the difference is likely very small, as synaptic and extrasynaptic channels are identical in cerebellar Purkinje cells (Hausser and Roth, 1997). Taken together, our work shows that DA activates D1-receptors to increase the phosphorylation on the AMPA channels, potentiating AMPA channel activity and thereby amplifying glutamatergic transmission from premotor interneurons onto motoneurons. This appears to be different to the mechanism of dopaminergic modulation in the lamprey spinal cord where D2 receptors contribute to the control of intrinsic excitability and afferent transmission (Schotland et al., 1995; Wikstrom et al., 1999; Svensson et al., 2003).

Our understanding of the actions of DA mechanisms within the spinal cord and particularly on motor systems is starting to grow (Clemens et al., 2006). In addition, to these effects there is also evidence that DA acting through D3 receptors inhibits afferent transmission, however in D3 knockout animals the effect switched to facilitation possibly via the D1-mediated mechanisms we observe (Clemens and Hochman, 2004). The actions of DA are complex and depend on the pre- and postsynaptic distribution of DA receptor subtypes, as well as rostrocaudal differences in receptor distribution. The functional effects of DA are known to be concentration dependent due to the differing binding affinities of the receptor classes. Indeed, DA release in the spinal cord may be modulated by circadian rhythms (Clemens et al., 2005). Finally, DA interacts with other monoaminergic systems to increase excitability (Madriaga et al., 2004) and bath application of DA and 5-HT has profound effects on locomotor circuits within the spinal cord (Kiehn and Kjaerulf, 1996; Schmidt and Jordan, 2000; Whelan et al., 2000; Gordon and Whelan, 2006). Clearly, more work needs to be performed to fully understand the role of DA and other monoamines within the spinal cord.

Acknowledgments—We greatly appreciate the technical assistance provided by Ms. Michelle Tran. This work was supported by an operating grant from the CIHR and ongoing support from the Alberta Heritage Foundation for Medical Research. Dr. Han received a PDF fellowship from the Hotchkiss Brain Institute.

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(Accepted 25 November 2008) (Available online 7 December 2008)