Dopamine Inhibits N-Type Channels in Visceral Afferents to Reduce Synaptic Transmitter Release Under Normoxic and Chronic Intermittent Hypoxic Conditions

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Dopamine inhibits N-type channels in visceral afferents to reduce synaptic transmitter release under normoxic and chronic intermittent hypoxic conditions. J Neurophysiol 101: 2270–2278, 2009. First published February 25, 2009; doi:10.1152/jn.91304.2008. Glutamatergic synaptic currents elicited in second-order neurons in the nucleus of the solitary tract (nTS) by activation of chemosensory and other visceral afferent fibers are severely reduced following 10 days of chronic intermittent hypoxia (CIH). The mechanism by which this occurs is unknown. A strong candidate for producing the inhibition is dopamine, which is also released from the presynaptic terminals and which we have shown exerts a tonic presynaptic inhibition on glutamate release. We postulated that tonic activation of the D2 receptors inhibits presynaptic calcium currents to reduce transmitter release and that in CIH this occurs in conjunction with an increase in the dopamine inhibitory response due to the increase in presynaptic dopamine receptors or an increase in dopamine release further suppressing the evoked excitatory postsynaptic current (eEPSC). Thus we predicted that blockade of the D2 receptors would return the EPSC to values of animals maintained under normoxic conditions. We found that dopamine and quinpirole, the selective D2-like agonist, inhibit calcium currents via the D2 receptors by acting on the N-type calcium channel in presynaptic neurons in their nTS central terminals. However, in brain slice studies from CIH animals, although the D2 antagonist sulpiride increased the CIH-reduced amplitude of synaptic currents, EPSCs were not restored to normal levels. This indicates that while the dopamine inhibitory effect remains intact in CIH, most of the reduction in the eEPSC amplitude occurs via alternative mechanisms.

INTRODUCTION

In previous studies, we demonstrated that dopamine, via the D2-like receptor, presynaptically inhibited the evoked excitatory postsynaptic current (eEPSC) between chemosensory and other visceral afferents and the secondary neuron in the nucleus of the solitary tract (nTS) (Kline et al. 2002). This synaptic current is mediated by glutamate acting on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Andresen and Yang 1990; Kline et al. 2002). Application of sulpiride, a D2-like receptor antagonist, increased the amplitude of the eEPSC via a presynaptic action demonstrating that dopamine has a tonic inhibitory effect on transmitter release (Kline et al. 2002). A potential mechanism for mediating the reduced transmitter release is a D2 receptor-activated block of presynaptic calcium channels. Previous studies by others have shown that dopamine can block T-, N-, P/Q-, and L-type calcium channel currents (Brown and Seabrook 1995; Cardozo and Bean 1995; Keja et al. 1992; Olson et al. 2005). The major calcium channel responsible for calcium influx at the presynaptic terminal in the nTS is the N-type calcium channel, with α-conotoxin GVIA blocking the majority of the eEPSC (Mendelowitz et al. 1995). These results suggest that the N-type channel is a likely target of D2 receptor activity. However, T-, P/Q-, and L-type are also present in the sensory neurons, providing additional D2 receptor-mediated targets.

Recently we reported that when rats are subjected to 10 days of chronic intermittent hypoxia (CIH) as a model of sleep apnea, the excitatory postsynaptic current in nTS diminishes (Kline et al. 2007). Because of diverse reports regarding the effects of hypoxia on D2 receptor transcription and on the expression of tyrosine hydroxylase (TH), the rate limiting enzyme for catecholamine synthesis (Bairam et al. 2003; Gozal et al. 2005; Huey and Powell 2000; Kobayashi et al. 1999), we postulated that tonic activation of the D2 receptors inhibits presynaptic calcium currents to reduce transmitter release and that in CIH, this occurs in conjunction with a rise in the dopamine inhibitory response, which is due to either the increase in presynaptic D2 receptors or an increase in dopamine release further suppressing the eEPSC. In the present study, we found that dopamine and quinpirole, the selective D2-like agonist, do inhibit calcium currents and stimulus-evoked changes in presynaptic calcium fluorescence via the D2 receptors by acting on the N-type calcium channel. However, in brain slice studies from CIH animals, we saw that although the D2 antagonist sulpiride increased the CIH-reduced amplitude of synaptic currents, EPSCs were not restored to normal levels. This indicates that while the dopamine inhibitory effect remains intact in CIH, much of the reduction in the eEPSC occurs via additional mechanisms.

METHODS

All experiments were performed on adolescent Sprague Dawley rats of either sex exposed to either room air or CIH. Hypoxic exposure began between the third and fourth week of age, and brain stem slices were generated following hypoxia. The Institutional Animal Care and Use Committee of Case Western Reserve University, University of Missouri and Pennington Biomedical Research Center approved all experiments and protocols.
Exposure to CIH

Exposure to intermittent hypoxia consisted of rats inspiring alternating cycles of 20.9 and 6% oxygen, nine episodes per hour, 8 h per day, as previously detailed (Kline et al. 2007). Briefly, standard rat cages containing unrestrained animals were placed in either an in-house made chamber or a commercially available hypoxic system (BioSpherix, Redfield, NY) for CIH exposure. The animals were fed ad libitum. The chamber was flushed with alternating cycles of pure nitrogen and compressed air (or pure oxygen) to reach the desired inspired oxygen levels. To achieve hypoxic levels, nitrogen was flushed in the chamber for 2 min. Oxygen levels decreased from 20.9% to 7% O2 within 80 s and remained between 6.5 and 6.2% for 50 s. Infusion of O2 subsequently increased chamber O2 to 20.9% within 2 min. O2 remained at this level for three additional minutes before the ensuing hypoxic exposure. The duration of the gas flow during each hypoxic and normoxic episode was regulated by solenoid valves controlled either by a timer or computer. Ambient oxygen, carbon dioxide, temperature and humidity levels were continuously monitored. Animals were subjected to CIH between 9:00 AM and 5:00 PM for 10 consecutive days. Between 5:00 PM and 9:00 AM animals were exposed to room air. Control (normoxic) animals consisted of rats not exposed to CIH. Animals were housed in standard rat cages placed in the vivarium, in the hypoxic chamber but only exposed to 20.9% O2 or, alternatively, on the top surface of the hypoxic chamber during littermate CIH exposure. All brain slice experiments or tissue harvesting were performed the morning after CIH exposure or the comparable normoxic time period.

Brain stem nTS slices

PREPARATION AND ELECTROPHYSIOLOGY. Brain stem slices containing the nTS were prepared from 5-wk-old rats following CIH or normoxia (Kline et al. 2002, 2007). Animals were anesthetized with Halothane (Halocarbon Laboratories, River Edge, NJ) and decapitated. The brain stem was removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). Horizontal slices (~300 µm) were cut with a sapphire knife (Delaware Diamond Knives, Wilmington, DE) using a vibrating microtome (Leica VT 1000S). Tissue sections were placed in a superfusion chamber, secured with a nylon mesh and superfused at a flow rate of 3–4 ml/min with ACSF at 31–33°C. All recordings were made from cells in the caudal nTS (medial and commissural subnuclei) that receives a high density of carotid body afferent fiber termination and cardiorespiratory innervation (Andresen and Kunze 1998), the antioxidant sodium metabisulfite (Na2S2O5, 75 µM, Sigma) was added to solutions containing DA (Behr et al. 2000; Kline et al. 2002). Bath solutions were delivered by gravity feed from 60-ml reservoirs bubbled with 95% O2-5% CO2. Switching among solution reservoirs occurred by the use of pinch valve controllers (Warner Instruments, Hamden, CT).

Isolated nodose/petrosal ganglia (NPG) neurons

CAROTID BODY CHEMORECEPTOR LABELING. In a subset of animals, the carotid body was labeled to study petrosal chemoreceptor sensory afferents. As previously described (Andrews and Kunze 2001), 21-day-old rats were anesthetized with isoflurane. A midline incision in the neck was made and the carotid body was located and separated from the surrounding tissue. The lipophilic dye, DiA (Molecular Probes, Eugene OR), was placed on the carotid body and sealed in place by Kwik-Sil (WPI). The wound was sutured and the rat allowed to recover. After 5 days of recovery with no observable adverse effects from the surgery, NPG cultures were generated.

NPG ISOLATION AND CULTURE. The ganglia were excised from 5-wk-old adult Sprague-Dawley rats, and neurons were isolated and cultured as previously described (Doan and Kunze 1999). Briefly, adult rats were anesthetized with Halothane (Halocarbon Laboratories, River Edge, NJ) and decapitated, and the ganglia were surgically removed. The ganglia were placed in cold nodose/petrosal complete media (NCM), which consisted of Dulbecco’s modified Eagle medium/F-12 (Gibco BRL), 5% fetal bovine serum (FBS, Cellgro, Herndon, VA), and 1% penicillin/streptomycin/neomycin (Gibco BRL). The ganglia were then placed in Earle’s balanced salt solution (Gibco BRL) containing 1 mg/ml collagenase type 2 (Worthington, Lakewood, NJ) and incubated at 37°C for 70 min. After incubation, the medium was replaced with NCM containing 1.5 mg/ml bovine albumin (Sigma, St. Louis, MO), and the tissue was triturated with a glass fire-polished Pasteur pipette to dissociate the cells. These were then placed into 35-mm Petri dishes and onto poly-d-lysine-coated coverslips. The cultures were maintained in a humidified incubator (5% CO2-95% air at 37°C).

ELECTROPHYSIOLOGY. Membrane currents were recorded using the patch technique for whole cell recording under voltage-clamp conditions. Data were digitized and analyzed using pClamp programs (Molecular Devices). Capacitance and series resistance compensation (50–70%) were applied. Electrodes (1–3 MΩ) were prepared from 7052 or 8161 glass (Garner Glass). Electrophysiological experiments were performed on isolated neurons from normoxic or CIH animals 4–24 h after plating. Experiments on cells isolated from CIH animals were performed within 4–24 h after the CIH protocol was complete. In each of the experiments, the current-voltage relationship was recorded followed by pharmacological manipulation of the current to identify its components.
VOLTAGE PROTOCOLS FOR CALCIUM CURRENTS. To examine the current-voltage relationship of the total Ca\(^{2+}\) current, the membrane potential was held at \(-80\) mV. A 30-ms prepulse to \(-100\) mV was followed by a series of depolarizing voltage steps in 10-mV increments from \(-70\) to \(+20\) mV for 400 ms at 0.2 Hz. The peak current during each step was used to plot the current–voltage relationship.

SOLUTIONS AND DRUGS. Recording electrodes were filled with (in mM) 124 CsCl, 2.2 EGTA, 0.2 CaCl\(_2\), 5 HEPES, and 2 MgCl\(_2\), pH adjusted to 7.2 with CsOH and 280–310 mosM. The extracellular bathing solution for voltage-clamp protocols contained the following (in mM): 139 TEACl, 2 BaCl\(_2\), 5.4 KCl, 10 glucose, 10 HEPES, and 5 4-aminopyridine (4-AP), pH adjusted to 7.4 with KOH. All drugs and blockers where diluted in the extracellular solution for voltage-clamp experiments as needed. (\(-\) )-Quinpirole HCl, (1 or 10 \(\mu\)M, Tocris, Ellisville, MO) was used either alone in extracellular solution or combined with a general calcium channel blocker, \(\omega\)-conotoxin GIVA and agatoxin (Sigma) and nimodipine (Miles Pharmaceutical, West Haven, CT) were either diluted in extracellular solution alone or with quinpirole or dopamine.

LOW-AFFINITY CALCIUM CHANNEL BLOCKERS. Dihydropyridine block of L-type channels is voltage dependent with the \(I_{\text{Ca}}\) value decreasing with depolarization. Cav1.2 is 90% blocked and Cav1.3 is 50% blocked at 1 \(\mu\)M at depolarized test potentials (Xu and Lipscombe 2001). We did not go above 1 \(\mu\)M because of potential effects of the dihydropyridine on other channels. \(\omega\)-conotoxin-GVIA blocks the \(N\)-type channel with an \(I_{\text{Ca}}\) of \(-0.7\) nM and a maximum block between 10 and 100 nM (Boland et al. 1994), and \(\omega\)-agatoxin IVA blocks P/Q channel with an \(I_{\text{Ca}}\) of 2–5 nM and full block at 100 nM (Mintz et al. 1992a,b; Venema et al. 1992).

Synaptic terminal calcium labeling and imaging in brain stem nTS slices

Imaging was performed as previously described (Rogers et al. 2006). Briefly, 4-wk-old rats were anesthetized with isoflurane and using aseptic techniques, and the NPG complex was exposed through a midline incision in the neck. A micropipette containing calcium green-1-dextran (15% with 1% Triton-X100 in distilled water) was connected to a Picospritzer (Model II, General Valve, Fairfield, NJ). On recovery from the anesthesia, animals were returned to room temperature until needed. The use of coronal sections from several animals allowed for the study of the role that quinpirole and \(\omega\)-conotoxin GIVA play in TS-induced changes in calcium fluorescence: (\%\(\Delta F/\Delta F_0\)) where \(F\) is the fluorescence intensity of the fluorescent varicosity field before stimulation and \(\Delta F\) is the change from this value during TS stimulation (Rogers et al. 2006). Background fluorescence was subtracted from \(\Delta F\) and \(F\). A responsive varicosity was classified as one in which TS stimulation produced a calcium signal elevation (\(\Delta F/\Delta F_0\)) of \(\geq 5\). The average of three TS-induced calcium changes that were separated by 30 s was taken as baseline control. Eight minutes following ACSF (time control), quinpirole, \(\omega\)-conotoxin GVIA, or quinpirole + \(\omega\)-conotoxin GVIA, TS stimulation was repeated. The values of the responses to the second TS-stimulation challenge (i.e., after drug treatment) were compared by ANOVA; Dunnett’s post hoc comparisons were made to the “time control” group that received only TS-stimulation a second time. Statistical significance was assigned to values of \(P < 0.05\).

RESULTS

Dopamine and the D2-like agonist, quinpirole, inhibit calcium currents in NPG neurons

Current-voltage relationships for calcium currents were obtained under voltage-clamp using barium as the ion-carrying species in adult NPG neurons. A second set of records was then obtained in the presence of 10 \(\mu\)M dopamine, followed by a wash. An example is shown in Fig. 1A. The membrane potential was held at \(-80\) mV, and 400-ms voltage steps were applied to elicit the current. The current–voltage relationship is illustrated in Fig. 1B. Most, but not all, cells expressed low-voltage-activated calcium current, and thus calcium currents were activated between \(-60\) and \(-50\) mV with the peak current occurring between \(-20\) and \(-10\) mV reflecting the relative contributions of the multiple channel types in the cell particular population. When present, the low-voltage-activated currents consisted of a transient T-type current evident at \(-50\) mV (Fig. 1A, arrow) and/or a more slowly inactivating nimodipine-sensitive current that is most clearly seen at the end of a depolarizing pulse to \(-50\) or \(-40\) mV (Fig. 1D, arrow). Neither low-threshold current was blocked by dopamine. In the presence of dopamine, the peak current decreased from 52 ± 8 to 39 ± 6 pA/pF, \(P < 0.02\) (Fig. 1B, n = 5). Two minutes of
wash returned calcium currents to those comparable to control. Using the same voltage-clamp protocol, we added the D2-like agonist quinpirole (10 μM) to the perfusate. Similar to dopamine alone, peak current decreased from 57 ± 6 to 42 ± 3 pA/pF (P < 0.02, n = 5, Fig. 1C). In a subset of carotid body-labeled petrosal neurons, peak current decreased from 57 ± 10.6 pA/pF in control to 46 ± 7.7 pA/pF in quinpirole (10 μM, paired t-test, P < 0.02, n = 8).

Block of N-Type calcium channels eliminates inhibition of calcium current by dopamine or quinpirole

To identify the specific calcium channels responsible for the effects of dopamine and quinpirole, we used three specific calcium channel blockers, ω-conotoxin GIVA (1 μM, N-type), nimodipine (1 μM, L-type), and ω-agatoxin IVA (0.5–1 μM, P/Q-type). In isolated nodose/petrosal neurons, N-type calcium currents represented, on average, 59.6 ± 5.1% of the total peak calcium current (n = 6), whereas L and P/Q-type channels represented 27.9 ± 4.1% (n = 8) and 13.1 ± 2.8% (n = 8) of the peak current.

Following ω-conotoxin exposure for 5–10 min to eliminate the N-type current, cells were impaled and perfused with a mixture of 1 μM ω-conotoxin (CTx) and either 10 μM quinpirole or 10 μM dopamine. In the presence of ω-conotoxin, dopamine or quinpirole did not block the remaining calcium current. Peak current was 25 ± 3 pA/pF in control versus 27 ± 2 pA/pF in the presence of dopamine (P > 0.05, n = 3) and 21 ± 2 pA/pF in control versus 20 ± 2 pA/pF in quinpirole (P > 0.05 n = 3, Fig. 2A).

In contrast to N-type channel blockade, in the presence of both L- and P/Q-type channels blockers, nimodipine (Nimo, 1 μM) and agatoxin-IVA (AgaTx, 0.5–1 μM), the ability of quinpirole to block peak calcium currents remained intact (from 34 ± 6 pA/pF in control to 22 ± 4 pA/pF in quinpirole, n = 4, P < 0.008, Fig. 2B). Interestingly, much of the sustained low-threshold calcium current was blocked in the presence of nimodipine which is consistent with the presence of Cav1.3 in these sensory neurons (Lipscombe et al. 2004).

In a subset of isolated NPG cells, in the presence of the N-type calcium channel blocker, peak calcium current reversibly increased from 21.4 ± 3.9 pA/pF in the ω-conotoxin control to 23.9 ± 4.5 and 35.1 ± 11.3 pA/pF after 30 s and 2 min, respectively, in the ω-conotoxin/quinpirole mix. Because of the large variability (range: 8–66%), the increase in current did not reach significance even at 2 min in our sample of 6 cells (P ≥ 0.17).

FIG. 1. Dopamine and quinpirole, a D2 receptor agonist, block calcium currents in nodose/petrosal neurons. A: currents elicited in response to a series of 400-ms, 10-mV depolarizing steps from −70 to +20 mV (−80 mV holding potential) are shown before, during, and following perfusion with dopamine. Note the rapidly inactivating T-type current elicited at low-voltage thresholds (arrow). B and C: the current-voltage relations obtained by plotting the peak current during each step against the voltage are shown for control and in the presence of 10 μM dopamine (B, n = 5) or the D2 agonist 10 μM quinpirole (C, n = 5). *, P < 0.05. D: protocol similar to that described in A. For clarity and to illustrate the presence of a noninactivating low-threshold current (arrow), only the current elicited at voltages between −70 and −40 mV are shown. This current is blocked by nimodipine (compare currents from −70 to −40 mV in A and B, dashed line box, in Fig. 2) and is indicative of Cav1.3 in these sensory neurons.
consistent with the notion that N-type calcium channels mediate synaptic transmission in the nTS and afferent somal calcium. Application of ω-conotoxin (CTx) decreased stimulus induced changes by 42.3 ± 10.2% (n = 4 slices, P < 0.001 vs. time control, ANOVA). In the presence of ω-conotoxin, quinpirole failed to attenuate calcium current further. Application of CTx prior to quinpirole resulted in a TS-evoked fluorescent decrease of 51.2 ± 12.2% (Fig. 3D), which was not significantly different from that of conotoxin or quinpirole alone.

Neurons isolated from 10-day CIH animals respond to quinpirole

In another set of experiments, the effect of quinpirole on calcium current in NPG neurons isolated from normoxic (control) and CIH animals were compared in paired experiments. CIH neurons showed a similar inhibition with quinpirole as compared with normoxic cells. In the presence of 10 μM quinpirole, the peak current was reduced from 59 ± 10 to 41 ± 6 pA/pF (n = 12, P < 0.01, paired t-test) following CIH as compared with 61 ± 5 to 46 ± 5 pA/pF (n = 12, P < 0.02, paired t-test) for normoxic. There was no difference between CIH and NORM responses (ANOVA with post hoc Tukey test, P > 0.05). Thus D2-like receptor activation inhibits the currents to a similar extent in the CIH animals as it does in the normoxic animals.

Dopamine modulates synaptic transmission in nTS from 10-day CIH animals

We asked whether the inhibition in calcium influx by dopamine observed in sensory afferents alters central neurotransmitter release similarly in both normoxic and CIH nTS brain slices. As previously reported by us (Kline et al. 2007), 10 days of CIH decreased TS-evoked EPSCs in nTS cells. Cells from normoxic animals averaged 180 ± 20 pA (n = 25 cells, 15 animals), whereas those from CIH animals were 100 ± 16 pA (n = 15 cells, 10 animals, P < 0.05, t-test). Synaptic jitter, one criterion for identifying a monosynaptic cell, was comparable between the two groups (normoxic, 0.107 ± 0.013 ms vs. CIH, 0.144 ± 0.013, P > 0.05, t-test). A minority of normoxic (n = 3, amplitude, 59 ± 9 pA; jitter, 0.509 ± 0.082 ms) and CIH cells (n = 2, amplitude, 76 ± 25 pA; jitter, 0.365 ± 0.020 ms) exhibited higher jitter values suggestive of polysynaptic cells and were not studied further.

In the majority of normoxic monosynaptic nTS cells (13 of 17), bath application of DA (100 μM, 5 min) induced a reversible decrease in the amplitude of TS-evoked EPSCs. EPSC amplitude averaged 193 ± 33 pA in control recordings and was reduced to 143 ± 27 pA in dopamine (P = 0.0001, paired t-test, 75 ± 5% of control, Fig. 4). Ten minutes of wash recovered EPSC amplitude to 200 ± 43 pA. Four of the 17 monosynaptic cells studied during dopamine exposure increased EPSC amplitude (214 ± 43 to 273 ± 73 pA, P > 0.05, paired t-test). Following CIH, dopamine (100 μM, 5 min) decreased EPSCs from 103 ± 9 to 85 ± 13 pA (79 ± 6% of control, P = 0.017, paired t-test, n = 7, Fig. 4). Ten minutes of wash recovered EPSC amplitude to 111 ± 11 pA. One CIH cell did not significantly change EPSC amplitude in response to dopamine exposure (61–66 pA). The decrease in EPSC amplitude to DA application was comparable between normoxic and CIH (P > 0.05, 2-way repeated-measures ANOVA).
We had previously identified the presynaptic D2-like receptor family as the primary mediator of DA's reduction on EPSC (Kline et al. 2002). Moreover, we showed that D2-like receptors tonically attenuate EPSC amplitude in that D2 blockade with sulpiride increased EPSC amplitude. We sought to determine if D2 blockade in CIH cells would restore EPSC amplitude to that of normoxic cells. Sulpiride (20 μM) alone increased EPSC amplitude from 135 ± 16 to 178 ± 21 pA, a 34 ± 9% increase in six of eight normoxic cells studied (P = 0.016, paired t-test on responsive cells, Fig. 4). Two cells did not respond to sulpiride application. Following CIH, sulpiride (20 μM) increased EPSC amplitude in five of eight cells from 64 ± 16 to 97 ± 23 pA, a 54 ± 19% increase (P = 0.021, paired t-test on responsive cells, Fig. 4). Three CIH cells did not respond to sulpiride application. The magnitude of increase in EPSC amplitude following sulpiride was comparable in cells from CIH and control (normoxic) animals (P = 0.3). Sulpiride also failed to increase EPSC amplitude in CIH exposed cells to those values seen in normoxic (control) slices.

**DISCUSSION**

The study makes two contributions to understanding the role of dopamine in synaptic transmission between sensory afferents and the secondary neurons in the nTS in both normoxic and chronic intermittent hypoxic conditions.

First, in sensory neurons isolated from the nodose/petrosal ganglia of animals exposed to either normoxia or CIH, dopamine acting through a D2 receptor inhibited N-type calcium currents. In nTS slices, the TS-evoked increase in calcium fluorescence was also attenuated by D2 receptor inhibition of N-type channels. Previous studies have shown that calcium influx derived from activation of the N-type channel is primarily responsible for the release of transmitter at the presynaptic
Dopamine modulates neuronal activity and synaptic transmission through D1- and D2-like receptors. In the nTS, only activation of D2-like receptors decreases synaptic transmission through a presynaptic mechanism (Kline et al. 2002). In the current study, we determined whether D2-like receptors modulate intracellular calcium in presynaptic sensory afferents as has been shown in other cell types (Missele et al. 1999). The D2 agonist quinpirole decreased calcium current and fluorescence in sensory somas and terminals, respectively. This occurred through inhibition of the N-type calcium channel. Blockade of P/Q- and L-type channels did not attenuate calcium current to dopamine application, confirming dopamine primarily modulates the N-type channel. The predominance of the N-type current in mediating NPG calcium entry is also in agreement with previous studies in these neurons (Mendelowitz et al. 1995). Moreover, the D2-induced reduction of synaptic transmission in the nTS (current study; Kline et al. 2002), which is mediated by N-type channels (Mendelowitz et al. 1995), is consistent with D2-like receptors reducing GABergic neurotransmission by blockade of N-type channels in the striatum (Momiyama and Koga 2001).

In a small subset of NPG neurons studied in culture, quinpirole increased calcium currents. Likewise, dopamine increased EPSC amplitude in a fraction of nTS cells. The increase in calcium currents and augmentation of EPSC amplitude in the presence of dopamine or quinpirole may occur through other members of the D2-like receptor family. The D2-like family consists of the D2, D3, and D4 receptor subtypes (Missele et al. 1999). Quinpirole is a D2-like receptor agonist able to bind to all members of the D2-like family. While the specific distribution of D2, D3, and D4 receptors in the rat nTS is unknown, these three receptors are widely distributed in the human nTS (Hyde 1996). Although the members of the D2-like family share similar homology and intracellular signaling, they may possess differing functional roles (Stanwood 2000). In medium-spiny GABAergic neurons and synapses of the nucleus accumbens, quinpirole increases intracellular calcium via D3 receptors and decreases calcium via D2 receptors (Mizuno et al. 2007). Altogether, while dopamine and quinpirole primarily decreased calcium currents as well as eEPSC amplitude, further studies are needed to ascertain whether D2 and D3 receptors differentially modulate intracellular calcium in NPG sensory cells and nTS.

Recently we reported that in nTS slices from rats subjected to 10 days of CIH there is a large reduction in EPSC amplitude (Kline et al. 2007). We hypothesized that D2 receptors are responsible for the attenuated current amplitude, and therefore blockade of these receptors would restore currents to control amplitudes. However, in nTS slice studies from CIH animals, although the increase in EPSC amplitude in the presence of sulpiride tended to be higher, the synaptic current was not restored to levels obtained in normoxia. Other factors must come into play.

FIG. 4. Dopamine inhibition is not altered in chronic intermittent hypoxia (CIH)-exposed slices. A: dopamine decreases excitatory postsynaptic current (EPSC) amplitude in cells from normoxic (left, n = 13) and CIH (right, n = 10)-exposed animals. *, P < 0.05, paired t-test. The decrease in EPSC amplitude to dopamine was comparable between normoxic and CIH cells. B: D2-like receptor blockade with the antagonist sulpiride (10 μM) increased solitary tract evoked EPSC amplitude in control (normoxic) and CIH-exposed cells. —, EPSCs in ACSF vehicle; - - -, EPSCs in sulpiride. Cells were held at −60 mV. Examples shown are an average of 15 current sweeps. C: control and CIH-exposed EPSCs during vehicle treatment were normalized to illustrate the comparable increase in currents to sulpiride (- - -) application. In B and C, stimulus artifacts were minimized for visual clarity. D: sulpiride increases EPSC amplitude in cells from normoxic (left, n = 5) and CIH (right, n = 5) exposed animals. *, P < 0.05, paired t-test. The increase in EPSC amplitude to dopamine was comparable between normoxic and CIH cells.

could block the D2 receptor with the antagonist sulpiride in jitter-identified second-order neurons in brain slices from normoxic or CIH animals. Results indicated that the tonic inhibitory effect on EPSC amplitude remains intact following CIH. Yet it does not account for the entire reduction in EPSC amplitude in CIH as the amplitude was not restored to levels obtained in normoxia. Other factors must come into play.

Dopamine modulates neuronal activity and synaptic transmission through D1- and D2-like receptors. In the nTS, only activation of D2-like receptors decreases synaptic transmission through a presynaptic mechanism (Kline et al. 2002). In the current study, we determined whether D2-like receptors modulate intracellular calcium in presynaptic sensory afferents as has been shown in other cell types (Missele et al. 1999). The D2 agonist quinpirole decreased calcium current and fluorescence in sensory somas and terminals, respectively. This occurred through inhibition of the N-type calcium channel. Blockade of P/Q- and L-type channels did not attenuate calcium current to dopamine application, confirming dopamine primarily modulates the N-type channel. The predominance of the N-type current in mediating NPG calcium entry is also in agreement with previous studies in these neurons (Mendelowitz et al. 1995). Moreover, the D2-induced reduction of synaptic transmission in the nTS (current study; Kline et al. 2002), which is mediated by N-type channels (Mendelowitz et al. 1995), is consistent with D2-like receptors reducing GABergic neurotransmission by blockade of N-type channels in the striatum (Momiyama and Koga 2001).

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DOPAMINE MODULATES N-TYPE CHANNELS IN CONTROL AND CIH

Dopamine receptor candidates for such reduction in synaptic transmission may include metabotropic glutamate (Chen et al. 2002) and GABA (Bailey et al. 2008) receptors, as well as many others (see Kline 2008). Alternatively, blood borne mediators, changes in intra-cellular messengers, channels and/or synaptic proteins may reduce neurotransmitter release (Kline 2008). The identity of this CIH-induced reduction in neurotransmission remains under investigation.

Within the nTS, dopamine and D2-like receptors likely play an important physiological role during CIH. During periods of intermittent hypoxia, such as those that occur during obstructive sleep apnea, increased circulating catecholamines, systemic hypertension, augmented basal ventilation and altered respiratory responses to hypoxia have been reported (Cistulli and Sullivan 1994). These physiological responses are due primarily to an augmentation of the peripheral carotid body chemoreflex (Fletcher 2001). Following CIH, an increase in DA content occurs in the carotid body (14 days of CIH) (Hui et al. 2003) and in the hypothalamus (35 of days CIH) (Li et al. 1996). Although we did not quantitatively examine dopamine levels within the nTS, a comparable rise in EPSC amplitude in response to D2 blockade, as well as the reduction of calcium currents in response to dopamine in NPG neurons, suggests CIH does not significantly alter dopamine signaling in the sensory afferent-nTS pathway studied in in vitro preparations. Whether DA modulates a reduction in nTS neurotransmission following CIH in intact preparations, which are not “washed out” unlike the current reduced approaches, will require additional experiments beyond the scope of this study.

In summary, dopamine acting via D2 receptors inhibits presynaptic N-type calcium currents in sensory afferent neurons inner-vating second-order neurons in the cardiorespiratory regions of the nTS. Dopamine released from presynaptic terminals acts in an autocrine fashion resulting in an inhibition of transmitter release. In spite of the documented changes in the dopaminergic system in various forms of hypoxia, at this particular synapse, the inhibition is comparable in animals exposed to either normoxic or chronic intermittent hypoxic conditions. The mechanism underlying the CIH reduction in evoked EPSCs cannot be attributed to changes in the dopaminergic system.

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