Dopamine Modulates Synaptic Transmission in the Nucleus of the Solitary Tract

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INTRODUCTION

The catecholamine dopamine (DA) modulates cardiorespiratory control via its actions in both the peripheral carotid body chemoreceptors and centrally in the brain stem. While the inhibitory actions of DA are well characterized within the carotid body (Gonzalez et al. 1994; Prabhakar 1994), the role(s) of DA in central respiratory control is less well defined. For instance, systemic administration of dopamine compounds that cross the blood-brain barrier suggest DA either increases (Hsiao et al. 1989; Huey et al. 2000a) or decreases (Bonora and Gautier 1988) respiration. Central administration of the non-selective DA agonist, apomorphine, also augments (Hedner et al. 1982) or attenuates (Bolme et al. 1977) respiration in the rat. In the in vitro neonatal rat brain stem-spinal cord preparation, dopamine increases respiratory motor output (Murakoshi et al. 1985). Thus whether central dopamine augments or inhibits respiration, and what are the site(s) of action, remain uncertain.

Cardiorespiratory reflexes during hypoxia originate primarily from the carotid body (Housley and Sinclair 1988). Chemoreceptor afferent fibers form synapses with second-order neurons in the central nucleus tractus solitarius (NTS) and are an important step in cardiorespiratory control (Andresen and Kunze 1994). A functional role for DA within the NTS is suggested by the presence of dopamine and its receptors in afferent fibers (Finley et al. 1992; Lawrence et al. 1995) and central NTS (Kalia et al. 1985; Kitahama et al. 2000; Yokoyama et al. 1994). Microinjection of DA in the NTS produces either pressor effects and tachycardia (Granata and Woodruff 1982) or depressor effects and bradycardia (Zandberg et al. 1979). During hypoxia, an increase in NTS DA concentration coincides with respiratory depression. The augmentation of DA levels can be eliminated following carotid sinus nerve sectioning (Goiny et al. 1991). Taken together, previous studies have suggested DA modulates cardiorespiratory function via its action(s) within the NTS. However, a direct understanding of the role for DA in synaptic transmission within this nucleus is lacking and was therefore examined in this study.

DA acts by binding to specific membrane receptors, which are divided into two families, D1- and D2-like (Missale et al. 1998). Direct activation of these receptors on the pre- or postsynaptic cell may significantly alter synaptic activity in the NTS. D2-like receptor protein and/or messenger RNA have been found in vagal (Lawrence et al. 1995) and petrosal (Czycz-Krzeska et al. 1992) afferent neurons. On the other hand, the presence and function of D1-like receptors in this region is controversial (Bairam et al. 1998; Czyzyk-Krzeska et al. 1992). D2-like receptors have also been localized to the caudal NTS (Huey and Powell 2000; Qian et al. 1997; Yokoyama et al. 1994), whereas D1-like receptors have yet to be found in the NTS (Qian et al. 1997). Therefore our main objectives are to determine whether DA modulates synaptic...
transmission in the NTS, and if so, what DA receptor subtype is responsible.

**METHODS**

**Afferent nerve labeling**

The Institutional Animal Care and Use Committee of Case Western Reserve University approved all experiments and protocols. Three-week-old rats were anesthetized with a cocktail containing 0.1 ml ketamine HCl (100 mg/ml), 0.1 ml xylazine (20 mg/ml), and 0.2 ml acepromazine maleate (10 mg/ml) at 1.2 ml/kg. The carotid body or vagus nerve was located and separated from the surrounding tissue. The lipophilic dye, DiA (Molecular Probes, Eugene, OR), was placed on the tissue and sealed in place by Kwik-Sil (WPI, Sarasota, FL). Previous studies by us (Mendelowitz et al. 1992) and others (Doyle and Andresen 2001) demonstrate that this procedure fills synaptic boutons belonging to the afferent fibers and that these boutons are concentrated in the caudal NTS. Animals were killed approximately 2 wk following dye application, and second-order cells exhibiting labeled synaptic boutons were examined in the isolated brain stem slice preparation.

**In vitro brain stem slice preparation**

Brain stem slices were prepared from 5- to 6-wk-old Sprague-Dawley rats (Zivic Miller, Pittsburgh, PA). Rats were deeply anesthetized with halothane and decapitated. The brain stem was removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing the following (in mM): 125 NaCl, 3 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 10 d-glucose, and 2 CaCl₂, saturated with 95% O₂–5% CO₂ (pH 7.4, 300 mosm). The medulla was trimmed ventrally to yield approximately 1.2 ml that contained inlet and outlet ports for ACSF flow. The submerged sections were secured with a nylon mesh and placed in ice-cold artificial cerebral spinal fluid (ACSF) containing medium was replaced with Dulbecco's Modified Eagle's medium/F-12 containing 5% fetal bovine serum, 0.1% sodium pyruvate, 1% penicillin/streptomycin, and 1.5 mg/ml albumin. The tissue was triturated to disperse the cells and placed into 35-mm Petri dishes containing poly-d-lysine–treated coverslips. Tissue was stored in a humidified incubator (5% CO₂, 37°C) for 1–2 days. Primary neuronal cultures were fixed at room temperature in 3% paraformaldehyde (30 min), washed in PBS, and exposed to PBS, 0.1% BSA, 0.01% saponin, and 1% donkey serum for 30 min. Cells were subsequently incubated for 1 6 h (4°C) with rabbit anti-D2 (1:750) and mouse anti-TH (1:200, Incstar) antibody in PBS containing 0.01% saponin and 1% BSA. After washing in PBS, sections were incubated for 90 min with Rhodamine X anti-rabbit IgG (1:500, Jackson ImmunoResearch Laboratories) in solution containing PBS, 10% donkey serum, 1.0% BSA, and 0.1% Triton X. Sections examined colocalization of D2 and synaptophysin were incubated for 90 min with Rhodamine X anti-rabbit IgG and anti-guinea pig biotin (1:200, Vector Laboratories), followed by FITC conjugated avidin (1:200, Vector Laboratories). Brain stem slices were mounted using Vectashield Mounting Medium containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories) and coverslipped. Sections were examined using a conventional microscope.

**Immunohistochemistry**

For localization of D2 receptors within the NTS, rats were anesthetized with halothane and decapitated. The brain stem was rapidly removed and trimmed rostrally and caudally to yield a 1.5-cm block centered on the obex. The tissue was embedded in tissue freezing medium (Fisher Scientific) and frozen in isopentane over dry ice. The brain stem was sectioned at 10–12 μm, placed on specimen slides, and fixed (5 min, 4°C) in 3% paraformaldehyde containing 0.1% Triton-X. Tissue sections were washed in PBS and subsequently exposed for 30 min to a PBS solution that contained 1.0% bovine serum albumin (BSA), 0.1% Triton, and 10% donkey serum. Sections were incubated (16 h, 4°C) with rabbit anti-D2 antibody (1:500, Chemicon International) and mouse anti-synaptophysin (1:500, Sigma, St. Louis, MO) or guinea pig anti-substance P (1:500, Incstar) in PBS, 0.1% Triton, and 1.0% BSA. After washing in PBS, sections examining the colocalization of D2 and synaptophysin were incubated for 90 min with Rhodamine X anti-rabbit IgG and FITC anti-mouse IgG (1:500, Jackson ImmunoResearch Laboratories) in solution containing PBS, 10% donkey serum, 1.0% BSA, and 0.1% Triton X. Sections examining colocalization of D2 and substance P were incubated with Rhodamine X anti-rabbit IgG and anti-guinea pig biotin (1:200, Vector Laboratories), followed by FITC conjugated avidin (1:200, Vector Laboratories). Brain stem slices were mounted using Vectashield Mounting Medium containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories) and coverslipped. Sections were examined using a conventional microscope.

**Drugs**

The following drugs were dissolved in ACSF and bath applied: dopamine-HCl (DA; Sigma), 6-cyano-7-nitroquinoxaline (CNQX; Tocris-Cookson, Ballwin, MO), (±)-1-phenyl-2,3,4,5-tetrahydropyridine-1-(H)-3-benzazine-7,8-diol (SKF 38393 HBr, a D1 agonist), (4AR-trans)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]-quinoline (quipinic; HCl, a D1 agonist), and (RSA)-(+)-5-amino-2-(phenylmethyl)quinolin-2-ol (BD120, a D2 antagonist). DA agonists and antagonists were purchased from Tocris-Cookson. To prevent the oxidation of DA, (Smythies and Galzigna 1998), the antioxidant sodium metabisulphite (Na₂S₂O₄, 75 μM, Sigma) was added to solutions containing DA (Behr et al. 2000). Bath solutions were delivered for 5 min by gravity feed from 60-ml reservoirs bubbled with 95% O₂–5% CO₂. Switching among bath solutions containing the above-mentioned drugs occurred with the use of valve controllers (Warner Instruments, Hamden, CT).
Neuronal recordings during the first 60 s were not included in the data analysis to compensate for dead space of the tubing between bath reservoirs.

Data analysis

Data were analyzed via Axon Clampfit and Synaptosoft Mini-Analysis Program (Decatur, GA) software. Synaptic latency and jitter were analyzed as recently described by Doyle and Andresen (2001). Briefly, latency was defined as the time between the onset of the stimulus artifact and the beginning of the excitatory postsynaptic current (EPSC). Jitter was calculated as the SD of the shock-to-shock variation in latency. Derived synaptic parameters such as peak amplitude (pA), decay time constant (τ, 10–90% of peak, ms), and frequency (Hz) were compared within groups using paired t-test. Each data point for a given trial was an average of 8–10 sweeps. Spontaneous EPSC (sEPSC) detection was set at 2.5 times the root mean-square noise level. sEPSCs with a peak amplitude smaller than 3 pA were rejected. Data were presented as mean ± SE. P values < 0.05 were considered significant.

RESULTS

Whole cell voltage-clamp recordings were used to examine the effect of dopamine (DA) on stimulus-evoked glutamate receptor-mediated EPSCs of caudal NTS (medial and rostral commissural subnuclei) neurons. These subnuclei receive considerable chemo- and baroreceptor innervation (Ciriello et al. 1994; Finley and Katz 1992). In a subset of animals, the presynaptic terminals were labeled by the lipophilic dye DiA. Neuronal recordings were made from 47 postsynaptic cells. DiA labeled synaptic boutons were associated with 16 of these cells, suggesting they were second-order neurons. An example of a NTS neuron with an attached fluorescent bouton(s) is shown in Fig. 1. In our preparation, cells were first examined under IR-DIC (Fig. 1A) and then under fluorescence (Fig. 1B). Neurons in which fluorescent bouton(s) were localized on IR-DIC visualized cells (Fig. 1C) were then examined under the whole cell voltage-clamp technique using a patch pipette (Fig. 1D).

Resting membrane potential (RMP) of NTS cells averaged −60 ± 2 mV (n = 47). Electrical shocks to the solitary tract (ST) evoked EPSCs that were abolished by the non-N-methyl-D-aspartate (NMDA) receptor antagonist CNQX (10 μM, n = 3, data not shown), as previously reported (Andresen and Yang 1990). The mean latency to EPSC onset was 3.9 ± 0.2 ms (n = 47). Of the neurons examined, the majority (n = 40, 16 of which were DiA positive) were considered monosynaptic as evidenced by the low jitter values averaging 66 ± 5 μs (Doyle and Andresen 2001). Of the monosynaptic cells, electrophysiological characteristics were comparable between the 16 DiA positive cells and the remaining 24 cells; therefore these data were pooled. The remaining cells (n = 7) did not exhibit DiA synaptic input and showed higher jitter (360 ± 62 μs), suggesting they were polysynaptic (Doyle and Andresen 2001).

Dopamine depresses evoked EPSCs

Bath application of DA for 5 min induced a reversible and dose-dependent alteration in the amplitude of ST-evoked EPSCs. Concentrations of 1 and 10 μM DA did not significantly alter EPSC amplitude (n = 9 each, 2 DiA labeled, P > 0.05, paired t-test). On the other hand, 100 μM DA significantly reduced EPSC amplitude (Fig. 2A). In control recordings, EPSC amplitude averaged 154 ± 20 pA. Bath application of 100 μM DA for 5 min significantly reduced EPSC amplitude to 113 ± 24 pA (Fig. 2B, 70 ± 5% of control, P < 0.01, paired t-test, n = 14, 3 DiA labeled). Ten minutes of wash recovered EPSC amplitude to 156 ± 29 pA. The mean decay time constant (τ) for EPSCs was fitted to a single exponential and was comparable between control and DA application (control τ10–90% = 5.2 ± 0.5 ms vs. DA τ10–90% = 5.6 ± 0.6 ms, P > 0.05, paired t-test). Decreases in EPSC amplitude after DA administration were not accompanied by alterations in input resistance (Fig. 2C, control; 631 ± 104 MΩ vs. DA; 662 ± 101 MΩ, n = 14, P > 0.05, paired t-test).

FIG. 1. Example of a fluorescent synaptic bouton on a caudal nucleus tractus solitarius (NTS) neuron. A: infrared-differential interface contrast (IR-DIC) image of a caudal NTS cell medial to the solitary tract. B: DiA labeling (green) of a synaptic bouton from a vagal afferent fiber was visualized under fluorescence. C: overlay of images from A and B illustrates the fluorescent bouton was on the soma of the NTS neuron. D: following identification of a bouton-labeled NTS cell, a patch electrode was guided to the cell under IR-DIC for whole cell voltage-clamp experiments. A–D, 63× objective.
DOPAMINE-MEDIATED INHIBITION IN THE NTS

Dopamine decreases EPSCs by a presynaptic mechanism

The mechanism by which DA exerts its effect on ST-evoked EPSCs may involve a decrease in presynaptic glutamate release, an alteration in postsynaptic glutamate receptors, or a combination of both. To investigate the pre- versus postsynaptic mechanism for DA’s depression of EPSCs, we examined the paired pulse depression ratio (PPD, with a fixed stimulus interval of 20 ms) of evoked EPSCs. In the PPD protocol, the relative size of the second EPSC is compared with the first EPSC [1−EPSC2/EPSC1 (Regehr and Stevens 2001)]. A change in the ratio of these amplitudes by DA, in the absence of changes in postsynaptic input resistance, is thought to reflect a presynaptic mechanism (Debanne et al. 1996; Zucker 1989; Zucker and Regehr 2002). As seen in Fig. 3A, two consecutive ST stimuli produced a significant reduction in the second evoked EPSC. This frequency-dependent depression in the NTS is primarily of presynaptic origin (Chen CY et al. 1999; Miles 1986; Schild et al. 1995). In control recordings, the amplitude of the EPSC1 was significantly greater than that of the EPSC2 (1st, 154 ± 20 vs. 2nd, 76 ± 14 pA, P < 0.01, t-test). In the presence of 100 μM DA, EPSC1 was reduced (70 ± 5% of control, P < 0.05, paired t-test), while EPSC2 was altered to a lesser degree (95 ± 12% of control, P > 0.05, paired t-test). The significant reduction in EPSC1, but not EPSC2, resulted in a decrease in the PPD ratio from 0.47 ± 0.07 during control recordings to 0.29 ± 0.09 during DA application (Fig. 3B, P < 0.05, n = 14, paired t-test). This suggests DA modulates synaptic activity by a presynaptic mechanism.

sEPSCs are attenuated by dopamine

sEPSCs within the in vitro NTS are derived primarily from local synaptic connections among neighboring cells (Fortin and Champagnat 1993). We subsequently examined whether DA alters sEPSCs and thus the local NTS network. Dopamine significantly decreased the frequency of spontaneous events from 9.4 ± 2.2 to 6.2 ± 1.4 Hz (n = 13, P < 0.05, paired t-test, Fig. 4A). Ten minutes of wash returned sEPSCs to control frequency of 9.9 ± 2.9 Hz. During control recordings, sEPSC amplitude ranged from 6.3 to 116.2 pA (13 cells, n = 1031 events), whereas during DA (100 μM), sEPSCs were between 5.8 and 107.5 pA (13 cells, n = 785 events, Fig. 4B). This change in sEPSC amplitudes produced a leftward shift in the cumulative probabilities of EPSC amplitudes (Fig. 4C). These results suggest that DA modulates spontaneous neuronal activity in the NTS.

D2-like receptors mediate the synaptic effect of dopamine

To identify the receptor subtype involved in DA’s action in the NTS, we performed a series of experiments using selective agonists and antagonists to mimic or block, respectively, DA’s effect on evoked EPSCs. Dopamine receptors can be subdivided into two pharmacologically and biochemically distinct classes, the D1- and D2-like receptors. The D1-like receptor agonist, SKF 38393 HBr, applied at 30 μM, had a small, insignificant effect on evoked EPSC amplitude (Fig. 5A, 93 ± 9% of control, P > 0.05, n = 5, 1 DiA labeled, paired t-test). In contrast, the D2-like agonist, quinpirole HCl, at 30 μM, produced a consistent decrease in EPSC amplitude to 73.4 ± 4.3% of control (Fig. 5B, P < 0.01, n = 8, 4 DiA labeled, paired t-test). The reduction in ST-evoked EPSC amplitude by quinpirole was not accompanied by alterations in input resistance or RMP (data not shown). These observations suggest that a decrease in EPSC amplitude by DA is mediated via D2-like receptors.
D2-like receptors. Consistent with a D2-mediated event, the D2 antagonist sulpiride (20 μM) prevented the DA-induced depression of EPSCs (Fig. 5C, 104 ± 9% of control, n = 7, 2 DiA labeled, P < 0.05, paired t-test). Moreover, sulpiride alone increased EPSC amplitude to 131 ± 11% of control (Fig. 5D, P < 0.05, paired t-test) in six of eight cells studied, including two that received DiA-labeled innervation. Alterations in sEPSCs were not observed during sulpiride administration (control recordings, 9.6 ± 1.4 Hz vs. sulpiride, 8.2 ± 1.2 Hz, P = 0.08, n = 8 cells). These findings suggest that in the NTS slice, DA tonically modulates sensory afferent-relay cell transmission, but not local spontaneous activity, through D2-like receptors.

Localization of D2 receptors in the NTS and afferent pathway

The results thus far suggest that D2-like receptors modulate synaptic transmission in the NTS by a presynaptic mechanism. To confirm the presence of D2 receptors at the NTS cell synapse, we examined caudal NTS neurons by immunohistochemistry. Investigation of the NTS identified D2 receptors in the caudal portion of the nucleus. To determine whether the observed receptors were either pre- or postsynaptic, we searched for colocalization of D2 with either synaptophysin, a presynaptic vesicle protein (Wiedenmann and Franke 1985) or substance P, a neurotransmitter released from baro- and chemoreceptor fibers (Gillis et al. 1980; Kalia et al. 1984). Indeed, D2 receptors were found with synaptophysin (Fig. 6A) and substance P (Fig. 6B). This suggests D2 receptors are presynaptic.

To confirm the notion that D2 receptors are presynaptic, we performed immunohistochemistry on cultured petrosal ganglion neurons that innervate the carotid body and send afferent fibers to the NTS. Chemoreceptor fibers were determined by the presence of tyrosine hydroxylase (TH) (Finley et al. 1992).
D2 receptors were readily found in cultured neurons that were negative as well as positive for TH. An example of D2 immunoreactivity in a TH positive cell is shown in Fig. 6C. These observations support the electrophysiological data and suggest presynaptic D2 receptors on chemoreceptor afferent fiber modulate DA’s actions in the NTS.

**DISCUSSION**

In this study we used pharmacological and electrophysiological techniques to examine the functional role of DA in the caudal NTS. Our results indicate that DA reduces excitatory synaptic transmission in a reversible manner. The modulation of EPSCs is mediated by tonically activated presynaptic D2-like receptors.

**Dopamine decreases synaptic activity in the caudal NTS**

The caudal NTS is the central relay site receiving visceral afferent fibers from baroreceptors, cardiac receptors, rapidly adapting lung stretch receptors, and carotid body chemoreceptors (Andresen and Kunze 1994). The area of the NTS we have focused on receives considerable carotid body innervation, as evidenced by immunohistochemical (Ciriello et al. 1994; Finley and Katz 1992) and electrophysiological (Chitravanshi et al. 1994) studies. The caudal NTS is vital in the control of respiration, such that kainic acid lesion of this region is highly effective in reducing the ventilatory drive during hypoxia in the rat (Housley and Sinclair 1988).

DA, DA receptors, and TH are localized in the peripheral (Czyzyk-Krzeska et al. 1992; Gonzalez et al. 1994; Prabhakar...
1994) and NTS (Huey and Powell 2000; Kalia et al. 1985; Kitahama et al. 2000; Lawrence et al. 1995; Qian et al. 1997; Yokoyama et al. 1994) components of the rat chemoreflex pathway. Peripherally, DA is released from the carotid body glomus cells to inhibit chemoreceptor sensory activity (Iturriaga et al. 1994). Within the petrosal ganglia, DOPA decarboxylase, the DA-synthesizing enzyme, is localized in the absence of the norepinephrine-synthesizing enzyme, dopamine β-hydroxylase (Finley et al. 1992), suggesting a portion of petrosal cells are dopaminergic. Within the NTS, DA-containing fibers are predominantly in the caudal two-thirds of the nucleus (Kalina et al. 1985). D2 receptors are localized to both pre- and postsynaptic cells in the medial NTS (Lawrence et al. 1995). Thus there is evidence for the presence of DA and DA receptors in the chemoreflex pathway. However, little is known regarding DA in NTS synaptic transmission. In the present study, DA dose-dependently and reversibly decreased the amplitude of ST-evoked EPSCs in the NTS. The action of DA is direct since ACSF containing only antioxidant did not alter synaptic activity.

**Dopamine mediates its effect through D2 receptors**

The DA receptor subtype that modulates neuronal activity may be either D1- or D2-like. Five DA subtypes have been found and grouped as D1-like (D1 and D5) or D2-like (D2, D3, and D4) based on their structural, biochemical, and pharmacological characteristics (Missale et al. 1998). Classically, D1-like receptors are coupled to the G protein, Gαs, and activate adenylyl cyclase, whereas D2-like receptors couple to Gαi and inhibit adenylyl cyclase (Missale et al. 1998). Our results suggest that the DA-mediated reductions in synaptic responses are likely to occur through D2 receptors. The D2 agonist quinpirole consistently and significantly decreased the amplitude of ST-evoked EPSCs, whereas the D1 agonist SKF 38393 had little effect. Moreover, the D2 antagonist sulpiride blocked DA’s modulation. The attenuation of glutamatergic transmission by D2 receptors has also been observed in other brain regions (Hsia et al. 1999; Hsu et al. 1995; Koga and Motomiya 2000) and confirmed in D2-deficient mice that exhibit facilitated glutamatergic transmission in the striatum (Cepeda et al. 2001).

The presence of D2 receptors in the caudal NTS (Fig. 6) supports a D2-mediated inhibition of synaptic transmission. D2 receptors were colocalized with synaptophysin, a presynaptic vesicle protein (Wiedenmann and Franke 1985) as well as substance P, which is from, in part, baro- and chemoreceptor fibers (Gillis et al. 1980; Kawano and Chiba 1984; Nagashima et al. 1989). These results suggest that D2 receptors are presynaptic. Such a notion for presynaptic location is substantiated by reports demonstrating D2 mRNA within the petrosal ganglion (Czyzyk-Krzeska et al. 1992) as well as identification of D2R in cultured TH positive presynaptic neurons (see Fig. 6C). D2 was also found in TH-negative cells, presumably other afferent fibers. Nonetheless, our results suggest D2 receptors are presynaptic and serve as autoreceptors to modulate afferent neuronal activity.

Interestingly, when the D2 antagonist sulpiride was given alone, a significant increase in EPSC amplitude occurred, suggesting D2 receptors in the NTS tonically modulate synaptic activity. A tonic D2-mediated attenuation of synaptic activity has been reported in other central synapses (Chen and Pan 2000; O’Donnell and Grace 1994; Ruel et al. 2001; West and Grace 2002), as well as in cell cultures (Sulzer et al. 1998). D2 receptors may attenuate glutamatergic transmission by altering one or more ion channels in pre- or postsynaptic cells. In presynaptic cells, DA may augment outward potassium current to hyperpolarize the cell and decrease calcium entry (Missale et al. 1998), resulting in a net reduction of glutamate release. Fass et al. (1999) recently demonstrated that DA, via D2 receptors, tonically inhibit L-type calcium currents. Whether such a tonic modulation of calcium and/or potassium currents occurs in petrosal presynaptic cells remains to be investigated.

**Tonic D2 inhibition of glutamatergic transmission may limit incoming afferent activity to the NTS and optimize information transfer to the CNS.** Single fiber recordings of rat chemoreceptor afferents during normoxia and hypoxia demonstrate activity ranges between 1 and 10 Hz (Vidruk et al. 2001). This range of activity would subsequently be processed within the CNS. However, within the NTS, as the frequency of afferent input increases, the postsynaptic response (e.g., EPSCs) progressively decreases. This frequency-dependent depression has been observed by many investigators (Chen CY et al. 1999; Doyle and Andresen 2001; Miles 1986), as well as us (Fig. 3), in the NTS. Liu et al. (2000) demonstrated that frequency-dependent depression is physiologically relevant in baroreflex control. These authors demonstrated by curve-fitting analysis that a 10% synaptic depression in the NTS allows for a 19% greater reflex output in sympathetic activity. Thus tonic inhibition of synaptic activity by D2-like receptors may serve to stabilize cardiorespiratory output during normoxia and fine tune respiration and blood pressure during hypoxic provocation. Such a notion is supported by recent studies that D2 mutant mice exhibit facilitated respiratory response to acute hypoxia (Huey et al. 2000b).

**Effect of dopamine on EPSCs is presynaptic**

We examined the alterations in the PPD ratio during DA application as an indication of a pre- or postsynaptic mechanism (Regehr and Stevens 2001; Zucker and Regehr 2002). In examining the PPD ratio, if DA acts presynaptically to reduce the probability of glutamate release from presynaptic terminals, then the ratio of the second EPSC to the first EPSC (EPSC2/EPSC1) amplitude should be altered. By contrast, if DA acts postsynaptically, the amplitude of EPSC1 and EPSC2 should be reduced to the same degree, and therefore their ratio will remain unchanged. Bath application of DA reduced EPSC1, with a lesser effect on EPSC2, to suggest DA promotes a decrease in presynaptic quantal release, rather than a decrease in postsynaptic glutamate sensitivity. This is substantiated during application of DA or D2 agonist by the lack of alterations in EPSC decay time constants or input resistance of the postsynaptic cell while membrane potential was clamped at −60 mV.

One or more mechanisms may be responsible for the reduction of transmitter release. The extent of EPSC depression depends on the number of vesicles in the reserve and release-ready pools and the transition of these pools in the synaptic bouton (Regehr and Stevens 2001; Schild et al. 1995). An increase in intracellular calcium accelerates the mobilization of vesicles from the reserve pool and facilitates depression recov-
ery (Zucker and Regehr 2002). If D2 receptors tonically attenuate calcium current, as suggested by Fass et al. (1999), depression of EPSCs by DA may be due to an alteration of calcium-dependent vesicular trafficking. Dopamine may also modulate the formation of the SNARE complex core, a calcium-dependent process that is essential for vesicular exocytosis (Chen YA et al. 1999). Following release of vesicle contents, the SNARE complex is recycled so that individual components can take part in another round of membrane fusion. Fisher and Braun (2000) demonstrated DA increases SNARE complex formation fourfold, which may reduce vesicle recycling.

Dopamine modulates spontaneous NTS activity

sEPSCs within the in vitro NTS are derived from local synaptic connections among neighboring cells (Fortin and Champagnat 1993). These sEPSCs may contain action potential-independent components, or miniature EPSCs, that are characteristic of spontaneous presynaptic neurotransmitter release. However, in the NTS, most sEPSCs (≥90%) are sensitive to tetrodotoxin, suggesting they are produced by action potential propagation from neighboring neurons within the slice (Fortin and Champagnat 1993). These authors suggested that groups of spontaneous EPSCs form a re-excitatory network responsible for the propagation of activity in the NTS. We therefore examined if DA attenuates spontaneous activity within the NTS as it does evoked activity. Application of DA significantly decreases the frequency of sEPSCs, suggesting DA not only inhibits synaptic activity but also the local network that functions within the NTS. Interestingly, D2-like receptors do not tonically modulate spontaneous EPSCs as they do evoked EPSCs in the slice. For instance, in presence of sulpiride, there was no significant alteration in sEPSC frequency. Dopaminergic modulation of the local network may originate from one or more terminals that enter the NTS from DA-rich brain stem areas, such as the area postrema (Andresen and Kunze 1994, Kalia et al. 1985) that may modulate the cardiovascular system (Ferguson 1991). The dopaminergic suppression of spontaneous EPSCs in the NTS may serve to repress local neuronal activity and action potential propagation from the NTS to other brain stem regions that modulate respiration and blood pressure.

In conclusion, we have demonstrated that DA depresses glutamatergic synaptic transmission in the caudal NTS via a presynaptic mechanism. The effect is specific, because the D2 antagonist blocks the attenuation of synaptic activity by DA, whereas the D2 agonist mimics this attenuation. Furthermore, D2-like receptors tonically modulate the synapse between sensory afferent fibers and caudal NTS cells. These results suggest DA in the chemoreflex pathway plays an important role in the modulation of respiration and blood pressure.

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