Catecholaminergic modulation of respiratory rhythm
in an in vitro turtle brain stem preparation

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Abstract—An in vitro brain stem preparation from adult turtles was used to determine effects of dopamine (DA) and norepinephrine (NE) on respiratory frequency in turtles, but the DA receptor agonist apomorphine (D1-D5) attenuates the DA-mediated increase in peak frequency by 52 and 59%, respectively. On the other hand, the DA-receptor antagonists apomorphine (D1-D5), quinuclidine (D2), and SKF-38393 (D1) had no effect on peak frequency. Prazosin, an α1-adrenergic antagonist (250 nM) abolished the DA-mediated frequency increase. Although NE (10-200 µM) and phencyclidine (10-200 µM, α1-adrenergic agonist) increased peak frequency from 0.6 ± 0.1 to 1.2 ± 0.3 peaks/min and from 0.6 ± 0.1 to 1.0 ± 0.2 peaks/min, respectively, these effects were not as large as that with DA alone. The data suggest that both dopaminergic and adrenergic receptor activation in the brain stem increase respiratory frequency in turtles, but the DA receptor-mediated increase is dependent on coactivation of α1-adrenergic receptors.

Keywords: dopamine; brain stem; norepinephrine; respiratory control; reptile; dopamine; brain stem; norepinephrine

In vertebrates, activation of peripheral and central nervous system (CNS) dopaminergic receptors modulates respiratory motor output. For example, dopamine (DA) is located within type I glomus cells of the carotid body and, when administered intravenously, depresses minute ventilation by inhibiting carotid body chemoreceptor activity (1, 2, 29). Although the modulatory actions of DA on respiration via effects on peripheral chemoreceptors are well studied, relatively little is known concerning how DA modulates respiratory motor output via effects on the CNS.

There is suggestive evidence that, within the CNS, endogenously released DA plays an important role in the modulation of respiration because DA-containing synaptic terminals are found in the dorsal and ventral respiratory groups of the medulla (7), as well as near respiratory motoneurons in the hypoglossal (28) and phrenic motor nuclei (14). In contrast to the inhibitory effects of DA at peripheral chemoreceptors, evidence suggests that activation of central DA receptors elicits complex effects on ventilatory activity. For example, intravenous apomorphine (a nonselective DA-receptor agonist that crosses the blood-brain barrier) nearly doubles respiratory frequency and phrenic motor output in carotid-denervated, vagotomized and anesthetized (19), or decerebrate dogs (20). Similarly, apomorphine injected into the fourth ventricle of anesthetized rats increases minute ventilation (~20% Ref. 12). On the other hand, other reports suggest that activation of central DA receptors may depress ventilation under conditions such as sustained hypoxia (11, 25, 26).

Although these studies provide evidence for modulation of respiratory motor output when central dopaminergic receptors are activated, they do not conclusively demonstrate a specific brain stem action (i.e., central vs. peripheral sensory receptors or brain stem vs. other CNS sites). An in vitro brain stem-spinal cord preparation from neonatal rats (18) provides more conclusive evidence that brain stem DA increases respiratory activity because bath application of DA increases the frequency of respiration-related discharge (~30%).

Although Murakoshi and colleagues (18) provide data supporting a stimulatory role of DA on brain stem respiratory motor output in the neonate, adult brain stem preparations have not been studied. Furthermore, the specific catecholaminergic receptor subtypes involved have not been investigated. Presently, at least five DA receptor subtypes have been identified and are classified into two subfamilies: D1-like (D1 and D5) and D2-like (D2, D3, and D4) (23). In addition, DA may activate adrenergic receptors either directly (at relatively high concentrations) or indirectly by being metabolized to norepinephrine (NE) (9). NE may also directly modulate respiratory motor output at both peripheral (21) and central (18) receptors. Therefore, our main objectives were to determine 1) how DA alters the frequency and pattern of respiratory motor output in an isolated adult vertebrate brain stem preparation; and 2) which dopaminergic and adrenergic receptor subtypes are involved. Thus experimental protocols were conducted to investigate the potential role of both dopaminergic and adrenergic receptors in modulating respiratory motor output. To achieve these objectives, we utilized an in vitro brain stem preparation from adult turtles (6, 16). Preliminary accounts of this work have been reported as an abstract (15).

METHODS

Adult turtles (Chrysemys picta), weighing 0.50 ± 0.30 kg, were obtained from local suppliers (Kons Scientific, Germantown, WI, and Lemberger, Oshkosh, WI) and kept in a large open tank with access to water, heat lamps, and dry areas. All surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee.
Turtle Brain Stem Preparation

Turtle brain stems were isolated as described previously (16). Briefly, turtles were paralyzed with pancuronium bromide (0.3–0.5 mg im) to facilitate orotracheal intubation. Shortly thereafter (5–10 min), an orotracheal tube was placed and the lungs were ventilated (10–15 breaths/min) with a mixture of halothane (4%) in 100% O2 for 10–15 min. In nonparalyzed turtles, this procedure resulted in loss of the corneal and head withdrawal reflexes within 1–2 min and limb withdrawal reflex to toe pinch within 3–4 min. The plastron was rapidly removed with an autopsy saw, and the ascending aorta was perfused with an ice-cold superfusate solution equilibrated with 95% O2-5% CO2 for 2 min. The solution composition was the following (in mM): 100 NaCl, 23 NaHCO3, 10 glucose, 5 HEPES (sodium salt), 5 HEPES (free acid), 3 KCl, 2.5 CaCl2, and 2.5 MgCl2 (pH 7.45). After decapitation and removal of the bone and muscle covering the brain stem dorsally, the preparation was submerged in ice-cold, oxygenated superfusate solution and dissected to leave intact the portion of the brain stem caudal to the optic lobes and cranial to spinal segment C1 (Fig. 1A). The tissue was pinned down on Sylgard (ventral surface up) in a recording chamber (10 ml volume) and bathed (2–3 ml/min) with superfusate solution flowing from a superfusate reservoir (500 ml). The tissue was allowed to recover and equilibrate for 60–90 min before a protocol was initiated. All experiments were performed at room temperature (referred to as 22°C).

Nerve Recording and Data Analysis

Respiratory activity was recorded with a glass suction electrode attached to XII nerve rootlets. Neural signals were amplified (>10,000) and band-pass filtered (10 kHz), using a differential alternating-current amplifier (model 1700, A-M Systems, Everett, WA) before being stored on magnetic tape by using a pulse-code modulation analog-to-digital converter (Vetter Instruments, Rebersburg, PA). Signals from the tapes were rectified and integrated (time constant = 200 ms) before being moving averaged (MA-821/RSP, CWE, Ardmore, PA) before being digitized and analyzed by using pCLAMP software (Axon Instruments, Foster City, CA).

Episode and peak variables were measured as shown in Fig. 1B. Two or more peaks separated by less than the
average duration of a peak within that recording were defined as an episode (16, 17). Episode frequency was defined as $1/T_{\text{epi}}$, where $T_{\text{epi}}$ is the time interval between the onset time of two sequential episodes. In cases where only single peaks were observed, the peaks were considered individual episodes with one peak per episode, and episode frequency was therefore equal to peak frequency (i.e., the time interval between the onset time of two sequential peaks; Fig. 1A). Neural minute activity was defined as the integrated discharge in arbitrary units per minute. In cases where the discharge pattern was not clearly separated into distinct peaks, a set of criteria was established as follows: 1) a burst of neural activity in the XII nerve root was defined as a peak if its amplitude and area were greater than 50 and 35% (respectively) of that for a typical (average) peak in the same recording; 2) within a prolonged set of bursts with multiple potential peaks (i.e., an episode), a burst had to meet the criteria stated above plus have the onset and termination of the burst be within 15% of the baseline before the burst was considered an individual peak; and 3) bursts not meeting the criteria for a peak were considered as extraneous discharges. Ten to twelve episodes were analyzed to obtain baseline data and data at each drug dose.

Experimental Protocols

Agonist studies. Baseline data were recorded for 20–40 min while the preparation was bathed with control superfusate solution. Agonists were added to the superfusate reservoir at increasing concentrations, beginning with 10 µM, followed by 50, 100, and 200 µM, and ending with a 45- to 90-min washout period. For each dose, time was allowed for the drug to pass from the reservoir to the chamber (5–10 min) before the respiratory output to reach steady state (10–20 min) before data were recorded for 5–40 min (10–12 episodes).

Antagonist studies. After baseline data were obtained, antagonists were added to the superfusate reservoir and 20–30 min were allowed for the drug to equilibrate in the chamber before new baseline data were recorded (20–40 min; 10–12 episodes) (Table 1). DA was then added to the superfusate reservoir as described above to complete a cumulative dose-response curve in the continual presence of the selected antagonist.

Drug List

The following water-soluble drugs were obtained from Research Biochemicals (Natick, MA).

Agonists.

Dopamine hydrochloride (3-hydroxytyramine hydrochloride)

R(-)-apomorphine hydrochloride [R(-)-10,11-dihydroxyapo-morphine hydrochloride; dopaminergic agonist]

(±)-SKF-38393 hydrochloride [(±)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrochloride; D1-dopaminergic agonist]

Quinlororine dihydrochloride (5αR-trans)-5,5a,6,7,8,9,9a,10-octahydo-6-propyl-pyrrolid[2,3-g]quinazolin-2-amine dihydrochloride; D2-dopaminergic agonist

Phenylephrine hydrochloride (α1-dopaminergic agonist)

Norepinephrine hydrochloride (adrenergic agonist)

Antagonists.

Eticlopride dihydrochloride [S(-)-3-chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)]-6-hydroxy-2-methoxy-benzamide hydrochloride; D2-dopaminergic agonist]

Prazosin hydrochloride (α1-adrenergic antagonist)

R(+)-SCH-23390 (R(+)-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrochloride; D1-dopaminergic agonist)

Statistical Analysis

All measurements are reported as means ± SE. Variables measured in arbitrary units (e.g., episode integral, neural minute activity, and peak amplitude) were normalized to baseline values. Statistical inferences concerning the effects of agonist concentration on respiratory variables were analyzed by using a one-way ANOVA with a repeated-measures design, followed by Student’s t-tests with the Bonferroni correction for multiple comparisons to identify values significantly different from baseline. Comparisons of response curves between agonists, or between agonists with and without the respective antagonists, were made with a two-way ANOVA with a repeated-measures design (Sigma Stat, Jandel Scientific Software, San Rafael, CA). P < 0.05 was considered significant.

RESULTS

The preparations (n = 36) used for our experiments generated stable respiration-related motor discharge under control conditions that was similar to that reported previously (16). Baseline data, separated by antagonist treatment group, before and after the addition of antagonists are shown in Table 1. On the addition of antagonists, no statistically significant differences were found between pre- and postdrug baseline values within any group, with the exception of significant differences that were present between the peak frequency (decreased) and rise time for peak amplitude (increased) for the pre- vs. postprazosin data (P < 0.05).

Table 1. Baseline values before bath application of drugs

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Episode Frequency, episodes/min</th>
<th>Episode Duration, s</th>
<th>Peak Frequency, peaks/min</th>
<th>Peak Rise Time, s</th>
<th>Peak Duration, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine response curve</td>
<td>0.57 ± 0.15</td>
<td>20.4 ± 2.9</td>
<td>0.89 ± 0.17</td>
<td>1.8 ± 0.3</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Norepinephrine response curve</td>
<td>0.45 ± 0.10</td>
<td>23.8 ± 3.9</td>
<td>0.48 ± 0.06</td>
<td>1.3 ± 0.2</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>Phenylephrine response curve</td>
<td>0.61 ± 0.11</td>
<td>26.8 ± 3.3</td>
<td>0.64 ± 0.09</td>
<td>1.4 ± 0.2</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>Pre-R[+]-SCH-23390</td>
<td>0.55 ± 0.15</td>
<td>32.4 ± 5.7</td>
<td>1.10 ± 0.50</td>
<td>2.0 ± 0.3</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>Post-R[+]-SCH-23390</td>
<td>0.58 ± 0.15</td>
<td>26.6 ± 2.0</td>
<td>1.20 ± 0.50</td>
<td>1.8 ± 0.5</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Preticlopride</td>
<td>0.38 ± 0.07</td>
<td>31.7 ± 8.0</td>
<td>0.38 ± 0.04</td>
<td>1.3 ± 0.2</td>
<td>4.6 ± 1.1</td>
</tr>
<tr>
<td>Posteticlopride</td>
<td>0.34 ± 0.07</td>
<td>29.3 ± 5.3</td>
<td>0.40 ± 0.04</td>
<td>1.4 ± 0.4</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>Preprazosin</td>
<td>0.51 ± 0.09</td>
<td>35.5 ± 6.7</td>
<td>0.55 ± 0.07</td>
<td>1.7 ± 0.4</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Postprazosin</td>
<td>0.31 ± 0.05</td>
<td>27.8 ± 4.1</td>
<td>0.39 ± 0.09</td>
<td>1.4 ± 0.2</td>
<td>6.3 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different (P < 0.05) from preprazosin value.
DA Increases Respiratory Activity

DA produced significant, dose-dependent increases in peak (burst) and episode frequencies (both $P < 0.05$; Figs. 1C and 2, A and C). In most preparations, the frequency increase began within 5 min of DA addition to the reservoir and progressively rose from $0.9 \pm 0.2$ to $2.4 \pm 0.3$ peaks/min and from $0.6 \pm 0.2$ to $1.7 \pm 0.2$ episodes/min. A few preparations ($n = 2$ of 6) exhibited an overshoot in peak and episode frequency before approaching a slower, steady-state frequency that was still above baseline at 15–20 min. Aside from this, no inhibitory effects on frequency were noted at any time.

DA significantly decreased peak amplitude up to $31 \pm 6\%$ ($P < 0.05$; Fig. 2D), with a corresponding $54 \pm 9\%$ reduction in episode integral ($P < 0.05$; Fig. 2B). Even though peak amplitude and episode integral significantly decreased with DA application, neural minute activity increased up to $99 \pm 9\%$ from baseline by 50–100 µM, reflecting the dose-dependent increase in peak frequency. Minute activity then decreased at higher DA concentrations because amplitude was depressed but frequency remained unchanged (Fig. 2E). Washout of DA restored most values to near baseline conditions, with the exceptions of peak amplitude and episode integral. No significant alterations were found in episode duration, number of peaks per episode, rise time for peak amplitude, or peak duration in response to DA administration (Table 2).

DA-Receptor Antagonists Attenuate DA-Mediated Effects

The D1-receptor antagonist R[+]SCH-23390 (10 µM, $n = 6$) partially but significantly antagonized the DA dose-response curve for peak (52%, $P < 0.05$), but not...
episode, frequency (Fig. 3, A and C). Although no statistically significant difference was present between the dose-response curves when the peak amplitude is compared, R[+]-SCH-23390 diminished the significance of DA-mediated decreases at 10–200 µM (i.e., there was a tendency toward partial antagonism of this effect; Fig. 3D). No other DA-mediated effects were significantly affected by R[+]-SCH-23390 (Fig. 3, B and E).

Application of the D2-receptor antagonist eticlopride (20 µM, n = 6) significantly attenuated the DA dose-response curves for both peak and episode frequencies by 59 and 56%, respectively (both P < 0.05; Fig. 3, A and C). Eticlopride also diminished the significance of the DA-mediated decrease in peak amplitude (only between 10 and 100 µM); however, there was no statistically significant difference between these curves overall (Fig. 3D). Eticlopride had no effect on the DA dose-response curve of any other variable (Fig. 3, B and E).

Do DA-Receptor Agonists Mimic the DA-Mediated Frequency Increase?

To further determine the role of dopaminergic vs. adrenergic receptors in the DA-mediated peak (burst)

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### Table 2. Variables not significantly altered by bath application of dopamine

<table>
<thead>
<tr>
<th>Dopamine Concentration, µM</th>
<th>Episode Duration, s</th>
<th>Peaks Per Episode</th>
<th>Peak Rise Time, s</th>
<th>Peak Duration, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20.4 ± 2.9</td>
<td>1.8 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>20.1 ± 2.1</td>
<td>1.5 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td>50</td>
<td>17.1 ± 1.2</td>
<td>1.5 ± 0.3</td>
<td>3.7 ± 0.5</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td>100</td>
<td>17.0 ± 2.1</td>
<td>1.6 ± 0.3</td>
<td>3.7 ± 0.5</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>200</td>
<td>16.6 ± 1.8</td>
<td>1.5 ± 0.2</td>
<td>4.0 ± 0.5</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>0/Wash</td>
<td>15.2 ± 1.7</td>
<td>1.3 ± 0.2</td>
<td>3.9 ± 0.3</td>
<td>7.5 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE.
frequency increase, we tested the effects of DA-receptor agonists. None of the agonists tested significantly affected peak (burst) frequency (Fig. 4), including 1) nonselective dopaminergic agonist apomorphine (0.1–100 µM; \( n = 5 \)), 2) D₁-dopaminergic agonist SKF-38393 (1–100 µM; \( n = 4 \)), or 3) D₂-dopaminergic agonist quinelorane (1–40 µM; \( n = 2 \)) did not significantly affect peak (burst) frequency. • baseline data (0 µM agonist).

DA-Mediated Frequency Increase Was Abolished by \( \alpha_1 \)-Adrenergic Antagonist

Bath application of the \( \alpha_1 \)-adrenergic antagonist prazosin (250 nM, \( n = 6 \)) decreased baseline peak frequency (0.55 ± 0.07 to 0.39 ± 0.09 peaks/min; Table 1) and completely antagonized DA-mediated effects on peak and episode frequency and on neural minute activity (\( P < 0.05 \); Figs. 5 and 6, A, C, and E). The episode integral and peak amplitude responses to DA were unaffected by prazosin (Fig. 6, B and D).

Does Adrenergic Receptor Activation Mimic DA Receptor Activation?

Because prazosin completely blocked the effects of DA on respiratory frequency, we tested the direct effects of NE and the \( \alpha_1 \)-adrenergic agonist phenylephrine on respiratory activity. NE (\( n = 6 \)) increased peak frequency from 0.5 ± 0.1 to 1.2 ± 0.3 peaks/min and episode frequency from 0.4 ± 0.1 to 1.1 ± 0.2 episodes/min (\( P < 0.05 \); Fig. 7, A and C). NE also significantly decreased peak amplitude at 100–200 µM (\( P < 0.05 \); Fig. 7D). These changes resulted in a significantly greater neural minute activity (to a maximum of 122 ± 43% above baseline) (Fig. 7E). The rise time to reach peak amplitude was also significantly increased between 50 and 200 µM with NE application (\( P < 0.05 \)), whereas DA application had no effect on this variable (data not shown). The episode integral was significantly diminished by NE only at 200 µM (Fig. 7B), whereas episode duration, peak duration, and number of peaks per episode were unchanged (data not shown). The NE dose-response curves were significantly less than those for DA in both peak and episode frequencies (both \( P < 0.05 \); 50 and 61% of the DA effects, respectively), but there were no overall differences in the dose-response curves of DA and NE for neural minute activity, episode integral, or peak amplitude (Fig. 7, A-E).

Bath application of the \( \alpha_1 \)-adrenergic-receptor agonist phenylephrine (\( n = 6 \)) significantly increased the peak and episode frequencies (significant only at 200 µM) from 0.6 ± 0.1 to 1.0 ± 0.2 peaks/min and from 0.6 ± 0.1 to 0.9 ± 0.2 episodes/min (\( P < 0.05 \); Fig. 7, A and C), respectively. Phenylephrine decreased the peak amplitude at 100 and 200 µM by a maximum of 20 ± 6% (Fig. 7D) and shortened the episode duration by 41 ± 8% (data not shown). The number of peaks per episode was also decreased by phenylephrine application at 10–200 µM, from 1.4 peaks/episode at baseline to 1.0 peaks/episode at all phenylephrine concentrations above 10 µM (data not shown). The neural minute activity (Fig. 7E) and other variables (i.e., episode duration, peak duration, and rise time to peak amplitude; data not shown) were not altered by phenylephrine administration. Figure 7, A-C and E, shows significant differences between DA and phenylephrine dose-response curves, with phenylephrine producing slower peak and episode frequencies (26 and 24% of the DA curve, respectively) and smaller neural minute activities and
DISCUSSION

The purpose of this study was to determine the effects of brain stem DA and NE on respiratory motor output and to characterize the receptor subtypes involved. The DA-mediated increase in peak and episode frequencies of respiratory motor output was partially attenuated by both D₁- and D₂-dopaminergic antagonists and was abolished in the presence of the α₁-adrenergic antagonist prazosin. Both NE and phenylephrine also increased the peak frequency, but not to the extent of DA. Because DA-receptor agonists alone had no effect on peak or episode frequency, and adrenergic agonists could account for only a portion of the DA-mediated effects, it appears that brain stem DA affects respiratory motor output through complex interactions between dopaminergic and adrenergic receptors.

Critique of Methods

The use of in vitro turtle brain stem preparations allowed us to assess central catecholaminergic effects in the absence of confounding peripheral sensory inputs in an adult, unanesthetized, aspirating vertebrate. Like isolated brain stems from other ectothermic vertebrates such as the frog (27), turtle brain stems are probably not hypoxic in the center and have a relatively smaller pH gradient compared with similar mammalian preparations. The turtle brain stem preparation has been shown to be remarkably insensitive to bath hypoxia and survives several days to weeks while

episode integrals (P < 0.05). No differences in dose-response curves were present in peak amplitude between drugs (Fig. 7D).

Fig. 6. Comparison of DA response curves with or without prazosin (250 nM). Prazosin abolished DA-mediated increases in episode frequency (A), neural minute activity (E), and peak frequency (C) but did not alter DA-mediated increases in episode integral (B) and peak amplitude (D). *Significantly different from baseline data (0 µM DA). †Statistically different response curve compared with DA curve (solid lines without symbols). Both P < 0.05.
producing respiratory motor discharge patterns similar to those in intact and vagotomized turtles (see Discussion in Ref. 16). Thus several unique features of this experimental preparation should be considered: 1) in our study of isolated brain stems, suprapontine and spinal neurons that may be activated by DA or NE and that project to the brain stem were eliminated; 2) the adult vertebrate brain stems used in our study avoid potential maturational changes in receptors (e.g., Ref. 10), although it may be that dopaminergic influences in adult mammals and adult turtles are different; and 3) the use of selective D1-, D2-, and α1-receptor agonists and antagonists allows an investigation of the relevant receptor subtypes in DA-mediated increases in peak or episode frequency.

Despite these advantages, there are also limitations to use of this preparation, some of which have been previously addressed (16). Specifically, the measured respiratory motor output originated from cranial nerve (XII) activity and not from nerves driving respiratory pump musculature. In turtles, XII nerve motor output is synchronous with respiratory airflow patterns (6), but it is unclear how measurements of this discharge correlate with tidal volume in chelonia. Thus peak and episode frequencies and the number of peaks per episode are the variables most likely to reflect changes in ventilatory activity in turtles. In addition, bath application of drugs limits conclusions concerning the specific brain stem sites of action.

Central Dopaminergic Modulation of Respiratory Activity

DA-mediated enhancement of respiratory activity. Ventilation increases after apomorphine injections into the fourth ventricle of rats (12), although others report a decrease in respiratory frequency after intracisternal apomorphine injections (3). When apomorphine is given

![Graphs showing effects of adrenergic agonists on respiratory discharge.](http://jap.physiology.org/)

Fig. 7. Effects of adrenergic agonists on respiratory discharge. Comparison of cumulative DA dose-response curves (solid lines without symbols) with response curves for norepinephrine (general adrenergic agonist) and phenylephrine (selective α1-adrenergic agonist). A, episode frequency; B, episode integral; C, peak frequency; D, peak amplitude; E, neural minute activity. *Significantly different from baseline data (0 µM). † Statistically different response curve compared with DA curve. Both P < 0.05.
intravenously to decerebrate, vagotomized, carotid-derervated dogs, phrenic motor nerve activity increases by ~200% because of increases in both the amplitude and frequency of discharge (20). Collectively, these experiments suggest that activation of central DA receptors alters respiration, but the location of the relevant DA receptors is uncertain. In an in vitro neonatal rat brain stem-spinal cord preparation, the frequency of respiratory discharge in spinal nerve C4 increased by 30% after bath application of 30 µM DA (18). Taken together, these data suggest that activation of brain stem DA receptors enhances ventilation in mammals.

Our results in turtles are similar to the abovementioned previous reports on mammals insofar as DA augments respiratory motor activity. However, unlike in mammals, DA-receptor agonists, such as apomorphine, did not evoke a similar response in turtles. Because, in in vivo mammalian studies utilizing apomorphine, a brain stem site of action could not be assured (20), and Murakoshi et al. (18) did not investigate the effects of DA-receptor agonists on in vitro respiratory motor output, it is not completely clear that turtles and mammalian species differ in any fundamental way. Nevertheless, the lack of evidence for a direct action of DA on respiratory motor output via dopaminergic receptors in an in vitro turtle brain stem suggests that the DA has no such direct effects or that the receptor pharmacology of turtle DA receptors is unique and is not responsive to the specific DA-receptor agonists chosen. On the other hand, both the D1- and D2-receptor antagonists (added separately) partially attenuate the DA-mediated peak frequency increase. Collectively, the data suggest that dopaminergic receptors play at least some role in the effect of DA on peak and episode frequency, although this effect must be complex because it appears to be contingent on coincident α1-adrenergic receptor activation.

DA itself can directly activate α- and β-adrenergic receptors (9), or DA may be converted to NE by dopamine β-hydroxylase, indirectly activating adrenergic receptors (4). In addition, activation of D2 receptors inhibits release of NE in rat frontal cortex (22), demonstrating that DA can also modulate the release of NE. These reports raise questions concerning the extent to which the DA-mediated effects may have been due to activation of adrenergic receptors in our study.

Is there a tonic DA-mediated stimulatory input to respiration? Previous studies show that ventilation decreases after administration of haloperidol intracerebroventricularly in anesthetized rats (12) or intravascularly in anesthetized dogs (19). A subsequent study in decerebrate dogs, however, showed no effect on ventilation after haloperidol administration, prompting the authors to hypothesize that decerebration interrupts suprapontine DA-sensitive pathways that tonically stimulate respiration (20). The findings in our study are consistent with this hypothesis because the D1- or D2-receptor antagonists had no effect on baseline values. Thus our results suggest that DA neurons found in the dorsal turtle brain stem (24) are either inactive or do not project to respiratory neurons. Bath application of prazosin, however, decreased baseline values of peak frequency (30%), suggesting that there may be a tonic, excitatory involvement of α1-adrenergic receptors in respiratory timing.

Modulation of respiration-related burst amplitude. In addition to changes in respiratory timing, certain features of the respiratory pattern such as peak amplitude and episode integral were decreased by DA. The explanation for these alterations is not clear. The DA-mediated decrease in peak amplitude tended to be diminished by R[−]-SCH-23390, although the response curves were not statistically different. D2-dopaminergic receptors are present on hypoglossal motoneurons in rats (28), and activation of these receptors by DA may have caused a decrease in peak amplitude. However, the inability of eticlopride to block the decrease is inconsistent with this idea. The role of α- and β-adrenergic receptors is also unclear because, although the adrenergic agonists decreased peak amplitude, the α2-adrenergic antagonist did not block this effect. If β-adrenergic activation by NE was responsible for the decrease, phenylephrine should have had no effect, which was not the case. Because the DA-mediated decrease in peak amplitude was not reversed during a lengthy washout period, it is possible that the amplitude decrease is attributable to decreased viability of the preparation over time, although this is unlikely because turtle brain stem preparations under similar conditions produce stable respiratory motor discharge with no changes in peak amplitude for up to 6 h (16). Thus the mechanism of decreased burst amplitude remains unclear.

Central Adrenergic Modulation of Respiratory Activity

NE modulates respiratory activity in vitro. The mechanism by which NE modulates respiratory frequency has been examined in studies similar to ours, but with mammalian preparations. In these studies, NE was bath applied to isolated in vitro brain stem-spinal cord preparations from neonatal rats. NE alters the frequency of respiratory discharge in these preparations, depending on the location of the rostral brain stem cut. In pontomedullary preparations (rostral cut at the collicular level), bath application of NE increases or decreases respiratory frequency (8). In nearly all medullary preparations (rostral cut at level of VI cranial nerve root), however, NE decreases respiratory frequency (13). On the basis of these and other data, it was hypothesized that noradrenergic neurons in the pons (area A5) project to the medullary respiratory rhythm generator, thereby decreasing respiratory frequency by an α2-adrenergic mechanism (8). Thus application of NE to medullary preparations activates α2-adrenergic receptors and decreases respiratory frequency. Application of NE to the entire brain stem, however, is thought to decrease the activity of the A5 noradrenergic neurons, releasing the medullary rhythm generator from α2-adrenergic inhibition of respiratory frequency and resulting in a net increase in burst frequency. This hypothesis was supported by experiments showing that pressure injection of NE into the caudal ventrolateral
pons immediately evoked an increase in respiratory frequency (13). In our turtle brain stem preparations, which included regions equivalent to the pons, adrenergic receptor activation by NE increased respiratory frequency, suggesting that the supramedullary influence of the respiratory rhythm generator is similar between adult turtles and neonatal rats.

Prazosin abolishes the DA-mediated frequency increase. One of the most intriguing and puzzling findings of this study was the observation that prazosin completely antagonized the DA-mediated increase in peak episode frequencies. This suggests that activation of α1-adrenergic receptors was necessary for the DA-mediated frequency increases but that α1-adrenergic receptor activation could not account for the full effect. The most likely explanation for such complicated results is that the DA-induced frequency increase is mediated by direct activation of dopaminergic receptors via a mechanism that requires coactivation of α1-adrenergic receptors.

Because neither NE nor phenylephrine increases peak frequency to the extent of DA, our hypothesis predicts that only coactivation of dopaminergic and α1-adrenergic receptors will express the full effect of DA on respiratory motor output (especially peak frequency). Similarly, complex interactions between DA and α1-adrenergic receptors have been previously reported. For example, the apomorphine-enhanced acoustic startle response in rats is also selectively blocked by prazosin (5). Thus similar DA and α1-adrenergic receptor interactions may be found in other regions of the CNS.

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