Serotonergic modulation of respiratory motor output during tadpole development

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Serotonergic modulation of respiratory motor output during tadpole development. J Appl Physiol 93: 936–946, 2002. First published May 31, 2002; 10.1152/japplphysiol.00104.2002.—To test the hypothesis that serotonin (5-hydroxytryptamine; 5-HT)-receptor activation elicits age-dependent changes in respiratory motor output, we compared the effects of 5-HT bath application (5-HT concentration = 0.5–25 μM) onto in vitro brain stem preparations from pre- and postmetamorphic bullfrog tadpoles. Recording of motor output related to gill and lung ventilation showed that 5-HT elicits a dose-dependent depression of gill burst frequency in both groups. In contrast, the lung burst frequency response was stage dependent; an increase in lung burst frequency at low 5-HT concentration (<0.5 μM) was observed only in the postmetamorphic group. Higher 5-HT concentrations decreased lung burst frequency in all preparations. Gill burst frequency attenuation is mediated (at least in part) by 5-HT1A-receptor activation in an age-dependent fashion. We conclude that serotonergic modulation of respiratory motor output 1) changes during tadpole development and 2) is distinct for gill and lung ventilation.

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MATERIALS AND METHODS

Experiments were performed on 58 bullfrog tadpoles (*Rana catesbeiana*) obtained from a commercial supplier (Charles D. Sullivan, Nashville, TN). Animals were housed in aquaria supplied with flowing, filtered, and dechlorinated Quebec City water maintained between 19 and 21°C (photoperiod = 12:12-h light-dark cycle). Tadpoles were fed a mixed diet of spinach and Nutrafin pellets for turtles and amphibians. All experiments complied with the guidelines of the Canadian Council on Animal Care. The institutional animal care committee approved the specific protocols used in this study.

In Vitro Brain Stem Preparations

Tadpoles were anesthetized by immersion in a solution of tricaine methane sulfonate (1:10,000) buffered to pH 7.8 with NaHCO$_3$. Once unresponsive to tail pinch, tadpoles were decerebrated by a transection just rostral to the eyes. Animals were then placed under the dissection microscope for determination of the developmental stage on the basis of the criteria of Taylor and Kollros (48) and were assigned to one of two groups: pre- (stages VI–XV; $n = 32$) or postmetamorphic tadpoles (stages XVI–XXV; $n = 26$). The cranium was opened to expose the brain stem and rostral spinal cord and to allow dissection of the cranial nerves. To reduce axonal conductance throughout the dissection procedure, the brain was irrigated with ice-cold (0–5°C) artificial cerebrospinal fluid (aCSF). The composition of the aCSF was identical to the one developed for tadpoles (28) and consisted of (in mM) 104 NaCl, 4 KCl, 1.4 MgCl$_2$, 10 d-glucose, 25 NaHCO$_3$, and 2.4 CaCl$_2$. The superfusate was equilibrated with a 98% O$_2$-2% CO$_2$ gas mixture and had a pH of 7.8. The brain stem was transected between the optic tectum and the forebrain and then caudal to the hypoglossal nerve before being transferred to a small petri dish coated with Sylgard (Dow Corning) where it was immobilized with insect pins. The arachnoid and pia membranes were carefully removed, and the brain was moved to the recording chamber (model RC 25, Warner Instruments) where it was placed ventral side up.

Electrophysiological Recordings

Bursts of respiratory-related motor activity were recorded simultaneously from the rootlets of the fifth and tenth cranial nerves with the use of suction electrodes. The pipettes were constructed from borosilicate glass (0.84 mm ID) pulled to a fine tip with a vertical microelectrode puller (Stoelting Instrument). The tip was broken and beveled to achieve appropriate tip diameter. Neural activity signals recorded from the suction electrodes were amplified (gain = 10,000) and filtered (low cutoff = 10 Hz; high cutoff = 1 kHz) by using a differential alternating-current amplifier (model 1700, A-M systems, Everett, WA). Vagal and trigeminal signals were then full-wave rectified and integrated (time constant 100 ms) by using a moving averager (model MA-821, CEW, Ardmore, PA). The raw and integrated nerve signals were viewed on an oscilloscope and digitized for recording with a data acquisition system (model DI-720, Dataq Instruments, Akron, OH). The sampling rate of the analog-to-digital conversion for the raw signal was 2,500 Hz.

Experimental Protocol

Once the recording electrodes were in place, the brain stem preparation was superfused with control (drug-free) aCSF at room temperature (20–22°C) equilibrated to pH 7.8 delivered at a rate ranging between 4 and 6 ml/min. The preparation was allowed to return to ambient temperature and stabilize for 45–60 min, until stable rhythmic neural activity was recorded from both nerves.

In the first series of experiments, the protocol began by the recording of baseline respiratory-related motor output for 10 min before 5-HT was added to a second aCSF reservoir (premetamorphic, $n = 14$; postmetamorphic, $n = 8$). The tadpole brain stem was exposed to the first 5-HT concentration for 10 min before a higher 5-HT concentration was delivered to the preparation. Other studies have shown that an equilibration period of at least 5 min is necessary to obtain measurements that do not reflect a transient effect of the drug (39). Brain stem preparations were exposed, in succession, to five increasing 5-HT concentrations: 0.5, 1.0, 5.0, 10, and 25 μM. The final bath application was followed by a “washout” period. The preparation was superfused with drug-free aCSF for a period ranging between 30 and 45 min before a final recording of respiratory-related motor output was made. Because 5-HT and several 5-HT active drugs are light sensitive, drug preparation and experiments were conducted with dim lights. Drug reservoirs were covered to minimize light exposure. All drugs were obtained from Sigma/RBI Aldrich (St. Louis, MO).

In a second series of experiments addressing the role of 5-HT$_{1A}$ receptors in the maturation of the respiratory control system, the effects of selective 5-HT$_{1A}$-receptor activation on respiratory-related motor output were compared between stage groups (premetamorphic, $n = 8$; postmetamorphic, $n = 10$). In these experiments, brain stem preparations were superfused with aCSF containing increasing concentrations of the high-affinity 5-HT$_{1A}$-receptor agonist (2)-8-hydroxy-2-di-(α-propylamino)tetralin hydrobromide (8-OH-DPAT; at concentrations of 0.5, 1.0, 5.0, 10, and 25 μM) according to the protocol described previously.

In this series, complementary experiments assessed the effects of 5-HT$_{1A}$-receptor activation in the presence of the selective, high-affinity 5-HT$_{1A}$ antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190; at concentration of 5 μM). Tadpole brain stems from each developmental group (premetamorphic: $n = 10$; postmetamorphic: $n = 8$) were superfused with NAN-190 for 10 min before 5-HT was added to the superfusate by using the protocol described previously.

Concentrations of 5-HT$_{1A}$ agonists and antagonists were chosen according to those used in other studies (1, 35, 39) and on the basis of preliminary experiments examining the dose dependence between age groups.

Data Analysis

Frequency and amplitude values for respiratory burst activity were obtained by analyzing the last 3 min of each 5-HT concentration (including baseline). In vitro tadpole preparations produce two patterns of respiratory-related neural activity: 1) high frequency, low amplitude, and 2) low frequency, high amplitude, reflecting fictive gill and lung ventilation, respectively (28, 49). Cranial nerve burst amplitude from a single electroneurogram is not always sufficient to adequately identify fictive gill and lung bursts (44). On the basis of the criterion proposed by Torgerson et al. (49), both nerve signals were analyzed simultaneously, and vagal nerve activity was used as a sensitive marker of fictive lung activity to distinguish between gill- and lung-related signals (26, 27). Gill and lung burst frequencies were obtained by counting the number of gill- and lung-related bursting events for the 3-min segment analyzed and were averaged for a 1-min period. Within a fictive breathing episode, the period between
two successive, uninterrupted lung bursts was measured. Each period value within the 3-min segment analyzed was then used to calculate the mean instantaneous lung burst frequency as an index of respiratory rhythm (23). The instantaneous frequency of the small-amplitude, gill-related movements was quantified the same way. In breathing pattern analysis, breathing episodes are designated on a somewhat subjective basis. Thus the number of fictive lung breaths within an episode was obtained by counting the number of large-amplitude lung bursts that occurred in succession with no pause longer than the length of two ventilation cycles between them (23). For both nerves, burst amplitude was measured at the highest point of the integrated discharge signal in arbitrary units and was normalized to the average burst amplitude recorded during the baseline period.

All measurements are reported as means ± SE. The results were analyzed statistically by using a two-way analysis of variance (Statview version 5.01, SAS Institute, Cary, NC) followed by Fisher’s protected least significant difference test (P < 0.05). A repeated-measures design was used when appropriate.

RESULTS

5-HT-induced Changes in Neural Correlates of Respiratory Activity in Pre- and Postmetamorphic Tadpoles

The trigeminal and vagal electroneurograms shown in Fig. 1 illustrate the differences in respiratory-related activity that were typically observed between brain stem preparations from pre- (A) and postmetamorphic tadpoles (B). Under baseline conditions (5-HT concentration = 0 μM; left), the most notable distinction between these recordings is the higher frequency of fictive lung ventilation in preparations from more mature animals. Fictive lung ventilations produced by brain stem preparations are grouped into episodes; however, the episodic nature of the respiratory motor output is more evident in brain stems from more advanced tadpoles where each fictive breathing episode usually consists of a cluster of two or more lung bursts.

Preparations from postmetamorphic tadpoles typically produced fewer gill-related (low-amplitude) bursts compared with preparations from less developed animals. Figure 1 also shows stage-related variations in the fictive respiratory response to 5-HT bath application at different concentrations. Detailed analysis of such recordings revealed the complex effects of 5-HT and 5-HT1A active drugs on the neural correlates of respiratory activity in this species.

Effects of 5-HT on fictive gill ventilation. As predicted from in vivo studies (5), mean baseline fictive gill ventilation frequency was slower in post- than premetamorphic tadpoles (Fig. 2A). Subsequent 5-HT bath application caused a dose-dependent decrease in gill burst frequency in both stage groups (Fig. 2A; P < 0.0001). Although gill burst frequency differed between groups, the effects of 5-HT on gill burst frequency were not stage dependent because analysis of variance revealed no significant between-factors interaction (5-HT concentration × stage group; P = 0.99). Expression of this variable as percent change from baseline provides an alternate way of comparing the effects of 5-HT between groups. This approach confirmed that, although the overall fictive gill frequency is less in postmetamorphic tadpoles, the fictive gill frequency responsiveness to 5-HT does not differ significantly between stage groups (Fig. 2B; P = 0.189). Instantaneous gill burst frequency did not differ between stage groups (P = 0.25), and it showed similar decreases after 5-HT bath application (Fig. 2C; P = 0.004).

In both groups, high 5-HT concentrations attenuated trigeminal gill burst amplitude (Fig. 2D; P < 0.0001).
Although the between-group differences are not statistically significant \((P = 0.62)\), the between-factors interaction indicates that 5-HT-induced attenuation of trigeminal gill burst amplitude is stage dependent \((P = 0.05)\). In both groups, trigeminal burst amplitude returned to baseline values at the end of the experiment \((\text{Fig. 2D}).\) 5-HT bath application exerted similar effects on vagal gill burst amplitude \(\text{(data not shown)}\). Because low-amplitude bursts \(\text{(gill related)}\) are not always present in vagal neurograms \(\text{(especially in more mature preparations)}\), the lower number of reliable recordings makes this analysis difficult.

**Effects of 5-HT on fictive lung ventilation.** Under all conditions, fictive lung ventilations were more frequent in preparations from postmetamorphic tadpoles \((\text{Figs. 1 and 3A}; P < 0.0001)\). This result differs from the instantaneous lung burst frequency, which was greater in less mature brain stems \((\text{Fig. 3C}; P = 0.0001)\). In the premetamorphic group, 5-HT concentrations \(\geq 5 \mu M\) decreased lung burst frequency below baseline value \((\text{Figs. 1 and 3, A and B}).\) This effect contrasts with the response observed in preparations from postmetamorphic tadpoles where \(0.5 \mu M\) 5-HT increased fictive lung ventilation frequency in most preparations \((5 of 8; 63\%\). Only 4 of 14 preparations \(28\%\) from less advanced tadpoles showed this increase. Subsequent application of higher 5-HT concentrations decreased fictive lung ventilation frequency in both groups \((\text{Fig. 3, A and B});\) these effects were reversed by the washout period. Analysis of variance revealed a between-factors interaction \(\text{(5-HT concentration } \times \text{ stage group): } P = 0.0005\), indicating that the effects of 5-HT on fictive lung breathing frequency are stage dependent. Again, expression of this variable as percent change from baseline confirmed that lung burst frequency responsiveness to 5-HT is stage dependent \((\text{Fig. 3B}; P = 0.014)\). Interestingly, 5-HT increased instantaneous lung burst frequency \((P = 0.0016)\), but there was no between-factors interaction \((\text{Fig. 3C}; P = 0.87)\).

Breathing pattern analysis showed that, under baseline condition, the mean number of lung bursts per episode in the premetamorphic group was nearly one-half the number of fictive breaths recorded in episodes of more mature preparations \(1.5 \pm 0.1 \text{ vs. } 2.6 \pm 0.3; P = 0.009; \text{Fig. 4A}).\) High 5-HT concentrations decreased the number of bursts per episode in the postmetamorphic group only \((P = 0.003; \text{Fig. 4A})\). As for lung burst frequency, the between-factors interaction confirmed that the effects of 5-HT on this variable are stage dependent \((P = 0.035)\).

Similarly, the baseline number of fictive breathing episodes per minute was greater in the postmetamorphic group \((P = 0.017; \text{Fig. 4B})\). 5-HT also reduced the number of episodes per minute \((P < 0.0001; \text{Fig. 4B})\), but the effects of 5-HT were not stage dependent \((\text{5-HT concentration } \times \text{ stage group: } P = 0.1)\). Both breathing pattern variables returned to pre-5-HT levels after the 30-min washout period \((\text{Fig. 4, A and B});\)
postmetamorphic groups, respectively). The responses were also similar between stage groups (pre- vs postmetamorphic: \( P = 0.74 \) and \( P = 0.27 \) for trigeminal and vagal neurograms, respectively).

5-HT-induced Changes in Respiratory-related Motor Output: Role of 5-HT\(_{1A}\) Receptors

Fictive gill ventilation. In preparations from younger tadpoles, bath application of 5 \mu M NAN-190 alone increased “basal” gill burst frequency (Fig. 6A); however, pretreatment with this 5-HT\(_{1A}\) antagonist did not prevent the decrease in fictive gill burst frequency after subsequent 5-HT bath application (5-HT concentration effect: \( P = 0.0001 \); Fig. 7A). This response was not different from control (\( P = 0.21 \)). In the premetamorphic group, application of the selective 5-HT\(_{1A}\) agonist 8-OH-DPAT decreased fictive gill burst frequency (\( P = 0.0004 \)) in a fashion similar to 5-HT (8-OH-DPAT vs 5-HT: \( P = 0.71 \)). The effects of both 5-HT\(_{1A}\) active drugs on instantaneous gill burst frequency paralleled the changes in gill burst frequency (data not shown). NAN-190 did not prevent the 5-HT-induced decreases in instantaneous gill burst frequency (5-HT effect: \( P = 0.01 \)). Moreover, activation of 5-HT\(_{1A}\) receptors with 8-OH-DPAT decreased instantaneous gill burst frequency more than with 5-HT alone (8-OH-DPAT vs 5-HT: \( P = 0.013 \)).

In preparations from postmetamorphic tadpoles, application of the same NAN-190 concentration had no significant effect on basal gill-related activity (Fig. 6A) but was sufficient to prevent 5-HT-induced decrease in

Fig. 3. Stage-dependent changes in fictive lung ventilation during 5-HT application at different concentrations. 5-HT bath application was followed by a washout period. Shown are the effects of 5-HT on 3 different variables: absolute lung burst frequency (A), change in lung burst frequency relative to pre-5-HT values (B), and instantaneous lung burst frequency (C). Each panel shows the response of the pre-\((n = 14)\) and postmetamorphic tadpoles \((n = 8)\). Values are means ± SE. *Value statistically different from pre-5-HT values (baseline), \( P < 0.05 \). †Value statistically different from corresponding values from the other stage group, \( P < 0.05 \).

Fig. 4. Changes in fictive lung breathing pattern after increase in 5-HT concentration in the artificial cerebrospinal fluid. 5-HT bath application was followed by a washout period. Shown are the effects of 5-HT on the number of lung burst per fictive breathing episode (A) and the number of fictive breathing episodes per minute (B). Each panel shows the response of the pre-\((n = 14)\) and postmetamorphic tadpoles \((n = 8)\). Values are means ± SE. *Values statistically different from pre-5-HT values (baseline), \( P < 0.05 \). †Value statistically different from corresponding values from the premetamorphic group, \( P < 0.05 \).
gill burst frequency (5-HT vs. NAN-190 + 5-HT; P = 0.001; Fig. 7). These results differ from those described previously for the premetamorphic group (stage effect: P = 0.013; compare Fig. 7, A and B).

8-OH-DPAT decreased gill burst frequency (P = 0.018), but the effect was less than with 5-HT alone (P = 0.05; Fig. 7B) and the change was less than in the premetamorphic group (stage effect: P = 0.03). There was no statistical interaction between factors (8-OH-DPAT concentration/stage: P = 0.29; compare Fig. 7, A and B). Similar effects were observed on instantaneous gill burst frequency: in the postmetamorphic group, NAN-190 effectively prevented the 5-HT-induced decrease in instantaneous gill burst frequency (5-HT concentration effect: P = 0.13), a response that differed from the premetamorphic group (stage effect: P = 0.0021; 5-HT concentration/stage: P = 0.011). 8-OH-DPAT elicited dose-dependent decreases in instantaneous gill burst frequency in this group (P = 0.032). Again, this response differed from the premetamorphic group (stage effect: P < 0.0001; 8-OH-DPAT concentration/stage: P = 0.98; data not shown).

With respect to the amplitude component, NAN-190 prevented changes in gill burst amplitude in both groups (Fig. 7, C and D; NAN-190 + 5-HT vs. 5-HT alone: P = 0.026 and P = 0.029 for pre- and postmetamorphic groups, respectively). 8-OH-DPAT had no effect in the premetamorphic group (P = 0.58), but it decreased trigeminal gill burst amplitude in more mature preparations (P < 0.0001). The between-factors interaction suggests that trigeminal gill burst amplitude responsiveness to 8-OH-DPAT is stage dependent (8-OH-DPAT concentration/stage group interaction: P = 0.0008; compare Fig. 7, C and D).

Fictive lung ventilation. In brain stems from younger tadpoles, application of NAN-190 had no effect on basal lung burst frequency (Fig. 6B). Subsequent application of low 5-HT concentrations in the presence of NAN-190 increased lung burst frequency. Application of 5-HT concentrations >5 μM depressed lung burst frequency (Fig. 8B). These effects differ from those of 5-HT alone (P = 0.0005). In the same stage group, 8-OH-DPAT increased fictive lung burst frequency (P = 0.067), a response that differed from the changes induced by 5-HT alone (P < 0.0001; Fig. 8, A and B). In the premetamorphic group, the addition of 5-HT in the presence of NAN-190 had no effect on instantaneous
lung burst frequency (NAN-190 + 5-HT vs. 5-HT alone: $P < 0.0001$); 5-HT$_{1A}$-receptor activation had no effect on this variable either (8-OH-DPAT alone: $P = 0.31$; data not shown).

In more advanced preparations, NAN-190 alone had no effect on lung burst frequency (Fig. 6B). Pretreatment with this 5-HT$_{1A}$ antagonist only prevented the facilitating effect of 5-HT (low concentration) on lung
burst frequency (5-HT vs. NAN-190 + 5-HT: $P = 0.0001$; Fig. 8C). This effect differed from the response of the premetamorphic group ($P < 0.0001$), and the between-factors interaction was significant ($P = 0.003$; compare Fig. 8, B and C). 8-OH-DPAT had no effect on fictive lung burst frequency ($P = 0.45$; Fig. 8, A and C), an effect that differed from the premetamorphic group ($P = 0.01$; compare Fig. 8, A, B, and C). The between-factor interaction was not significant ($P = 0.28$). 5-HT$_{1A}$-receptor inactivation before 5-HT bath application had no significant effect on instantaneous lung burst frequency (5-HT vs. NAN-190 + 5-HT: $P = 0.33$). 8-OH-DPAT had no significant effect on this variable ($P = 0.31$; data not shown).

NAN-190 did not prevent 5-HT-induced attenuation of trigeminal lung burst amplitude (NAN-190 + 5-HT vs. 5-HT: $P = 0.656$ and $P = 0.437$ for pre- and postmetamorphic groups, respectively; Fig. 9, A and B). 8-OH-DPAT attenuated trigeminal lung burst amplitude similarly in both stage groups (pre- vs. postmetamorphic: $P = 0.998$), and in a manner comparable to that of 5-HT alone (8-OH-DPAT vs. 5-HT: $P = 0.20$ and $P = 0.64$ for pre- and postmetamorphic groups, respectively). These effects were similar for vagal lung burst amplitude (Fig. 9, C and D).

**DISCUSSION**

We compared the effects of 5-HT bath application on the neural correlates of respiratory activity between pre- and postmetamorphic tadpoles. To the best of our knowledge, this study is the first to address developmental changes in serotonergic modulation of respiratory activity with the use of a single experimental model. Our results clearly show that the effects of 5-HT on respiratory motor output 1) change during tadpole development and 2) differ between gill- and lung-related activity. The demonstration that 5-HT$_{1A}$ active drugs produce complex, stage-dependent changes in lung burst frequency suggest, albeit indirectly, that changes in 5-HT$_{1A}$-receptor expression may contribute to the increasing expression of fictive lung ventilation during tadpole development.

**Critique of Methods**

5-HT affects many types of neurons throughout the brain stem, thereby making it difficult to determine the specific site(s) of action of the drugs added to the superfuse. Moreover, it is possible that, in amphibians, medullary raphe neuron activity is regulated by a mammalian-like mechanism involving presynaptic 5-HT$_1$ autoreceptors (15, 51). This implies that changes in respiratory motor output after pharmacological activation or inactivation of 5-HT$_{1A}$-receptor subtypes may reflect changes in endogenous 5-HT release rather than activation or inactivation of postsynaptic mechanisms. Furthermore, the selection of pharmacological tools was on the basis of information derived from mammalian studies. Because the extent to which bullfrog 5-HT-receptor subtypes differ from mammals is not known, we assumed that the affinity and specificity of the drugs used applied to our model. Consequently, drugs may interact with 5-HT-receptor subtypes other than...
those targeted, or even with other neurotransmitter systems either directly (acting on nonserotonergic receptors) or indirectly, because of the extensive interactions between serotonergic raphe neurons and other neurotransmitter systems (e.g., dopaminergic or noradrenergic). Despite these limitations, the sum of our data supports the amphibian brain stem preparation as a valuable experimental approach for in vitro investigation of the development of the respiratory control system. Several valuable conclusions concerning developmental changes in serotonergic modulation of the respiratory control system can be drawn from the present study.

Developmental Changes in Serotonergic Modulation of Fictive Gill Ventilation

Results show that 5-HT depresses low-amplitude, gill-related activity in a dose-dependent fashion and that the gill burst frequency responsiveness to 5-HT does not change during development. The parallel decreases in instantaneous burst frequencies indicate that 5-HT-induced depression of the underlying respiratory rhythm contributes to the frequency response similarly in both stage groups. The group differences in gill burst frequency can then be related to 1) more frequent lung bursts in more advanced tadpoles and 2) the enhanced burst amplitude sensitivity to 5-HT in the postmetamorphic group (discussed below). The latter effect made it difficult to detect gill-related activity at high 5-HT concentrations and thus biased mean frequency values toward zero.

Bath application of 5 µM NAN-190 increased basal gill burst frequency in premetamorphic tadpoles only. Moreover, this pretreatment was sufficient to prevent gill burst frequency depression in the postmetamorphic but not the premetamorphic group. These results suggest 1) endogenous 5-HT is released in this preparation, 2) tonic inhibition of gill activity occurs during early development, 3) 5-HT1A-receptor activation contributes to this attenuation, and 4) 5-HT1A-receptor density on neurons associated with gill rhythm generation may be greater in the premetamorphic stages. The greater 8-OH-DPAT frequency effects in the premetamorphic group corroborate this interpretation. The fact that 8-OH-DPAT depressed fictive gill frequency less than 5-HT alone in the postmetamorphic group suggests that other mechanisms, such as emergence of other receptor subtypes and/or developmental changes in serotonergic innervation, may be involved.

5-HT application elicited dose-dependent attenuation of gill burst amplitude also. However, between-group comparisons showed that, unlike the frequency response, trigeminal gill burst amplitude responsiveness to 5-HT increases during development. This stage-dependent effect is likely mediated (at least in part) by 5-HT1A-receptor activation because in both stage groups NAN-190 prevented 5-HT-induced attenuation of trigeminal gill burst amplitude. Because 5-HT1A-receptor expression on motoneurons changes during development (46, 47), the stage-dependent effects of 8-OH-DPAT on trigeminal gill burst amplitude may reflect an increase in 5-HT1A-receptor density on these motoneurons. But the fact that 8-OH-DPAT-mediated attenuation of trigeminal lung burst amplitude (same motor pool) is not stage dependent does not support this hypothesis. Consequently, the mechanisms responsible for developmental enhancement of responsiveness to 5-HT1A activation likely act at a different (premotor) site. The lack of neurophysiological data on 5-HT modulation of gill ventilation makes it difficult to propose more explanations for these results.

Developmental Changes in Serotonergic Modulation of Fictive Lung Ventilation

In premetamorphic tadpoles, fictive lung ventilation response to 5-HT was similar to the changes in gill-related activity discussed previously. That is, a dose-dependent depression of burst frequency and amplitude notable mainly at 5-HT concentrations >5 µM. In the postmetamorphic group, however, low 5-HT concentrations increased, whereas higher concentrations decreased lung burst frequency. These important differences in the frequency response at lower 5-HT concentrations are at the basis of the stage-dependent differences in lung frequency responsiveness to 5-HT. Because the frequency increase occurred at 5-HT concentrations that correspond to endogenous 5-HT overflow concentrations measured in mammals by chronocardiometry [in vivo (21, 29)] or fast cyclic voltammetry [in vitro (38)], we hypothesize that the increase in lung burst frequency represents the physiological response to raphe neuron activation.

The variability of the breathing pattern data does not allow us to ascribe the frequency increase to one variable in particular. It appears that the lung burst frequency increase is the product of marginal changes in the number of fictive breathing episodes per minute and the number of lung bursts per episode. These changes in breathing pattern are small, but, given the episodic nature of lung ventilation in this species, they are sufficient to supersede the contribution of instantaneous lung burst frequency as determinants of the overall fictive lung ventilation frequency.

5-HT1A active drugs produced complex, stage-dependent changes in lung burst frequency, suggesting that opposing 5-HT influences modulate this aspect of respiratory motor output. On the basis of mammalian pharmacology, 8-OH-DPAT would shift this balance by reducing both endogenous 5-HT release (because of raphe autoreceptor activation) and the ensuing tonic activation of non-5-HT1A receptors. In contrast, blockade of 5-HT1A receptors during 5-HT application would reveal the influence of other 5-HT-receptor subtypes on this variable. Our results show that, at low agonist concentration, 5-HT1A-receptor activation stimulates lung burst frequency in a manner similar to the effect of 5-HT observed in the postmetamorphic group. The absence of a significant lung frequency increase with 8-OH-DPAT application in the postmetamorphic group implies that, in more advanced tadpoles, other receptor
subtypes contribute to the stimulatory effect of 5-HT on lung ventilation. In that regard, Johnson et al. (19) recently showed that 5-HT₃-receptor activation increases fictive breathing frequency in brain stem preparations from mature turtles.

The absence of developmental data for a single model system makes it difficult to compare our results with other studies. However, fictive lung ventilation data from premetamorphic tadpoles are consistent with the 5-HT₁A-mediated excitation of respiratory frequency reported in fetal and neonatal rats (8, 9, 33, 34, 37); for review, see Ref. 14]. Conversely, data from postmetamorphic tadpoles are consistent with 5-HT₁A-mediated depression of lung burst frequency and amplitude in mature turtles (19).

Respiratory Rhythm Generation and Development

The instantaneous lung burst frequency reported for the postmetamorphic group is similar to what has been reported previously for intact (23, 24) and decerebrate bullfrogs (25). The absence of stage-related differences for the instantaneous gill frequency data, with and without 5-HT, suggests that the mechanisms underlying the generation of this respiratory rhythm do not change during development. On the other hand, the instantaneous lung burst frequency data suggest that the intrinsic rhythm for lung burst generation is faster during early development. In both cases, however, the effects of 5-HT did not differ between groups, indicating that developmental changes in serotonergic modulation do not take place at this level. The reasons underlying such changes in lung-related rhythm generation remain unknown, but they may be related to the rostrocaudal translocation of the lung rhythmic mechanisms that take place during tadpole development (45, 50). These differences, along with the opposing effects of 5-HT on instantaneous gill and lung burst frequencies, further support the hypothesis that gill and lung ventilations are generated by distinct neural mechanisms (11, 20, 23) and that they are modulated differently.

Perspectives

This study showed that the changes in respiratory motor output that characterize tadpole development are associated with significant alterations in the effects of 5-HT bath application on the neural correlates of ventilation. The differences between gill- and lung-related activities, especially with respect to frequency, indicate that the neural mechanisms controlling these two modes of breathing are substantially different. The results of experiments that used selective agonist and antagonist are consistent with our initial hypothesis that 5-HT₁A-receptor subtypes contribute to the stage group differences in 5-HT responsiveness for specific components of respiratory motor output. However, the widespread action of the agents used limits our interpretation of the data.

Do changes in the serotonergic system contribute to the transition from aquatic to predominantly aerial respiration in this model system? Although it is clear that our study alone cannot answer this question, the demonstration that bath application of physiologically relevant concentrations of 5-HT facilitates the expression of fictive lung ventilation justifies the pursuit of other complementary experiments.

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