Reflex interactions between aerial and aquatic gas exchange organs in larval bullfrogs

NIGEL H. WEST AND WARREN W. BURGGREN
Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada; and Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01003

WEST, NIGEL H., AND WARREN W. BURGGREN. *Reflex interactions between aerial and aquatic gas exchange organs in larval bullfrogs.* Am. J. Physiol. 244 (Regulatory Integrative Comp. Physiol. 13): R770–R777, 1983.—The interaction between lung and gill ventilation was investigated in unanesthetized *Rana catesbeiana* larvae (stage XVII–XIX) in hypoxic (P_{O_2} = 80 ± 1 Torr), normoxic (P_{O_2} = 142 ± 1 Torr), and hyperoxic (P_{O_2} = 580 ± 23 Torr) water. Lung inflation with N_2, air, or O_2 initially reduced the frequency (f_G) and buccal pressure amplitude (P_{BC}) of the following gill ventilation cycles. However, 65 s after lung inflation f_G was significantly greater for N_2 inflation than for air inflation, whereas f_G after O_2 inflation was significantly lower. In further experiments, the effects of lung inflation and gaseous P_{O_2} on gill ventilation were dissociated. Flow of N_2, air, or O_2 was established through the lungs of tadpoles in hypoxic water. Flow of N_2 produced a significant rise in f_G and flow of O_2 a significant fall compared with airflow. Regardless of the P_{O_2} of the gas, subsequent lung inflation produced by occlusion of the outflow cannula caused a fall in f_G and P_{BC} from the preestablished flow value. We conclude that lung inflation per se and the resulting increase in P_{O_2} both contribute to the suppression of gill ventilation following spontaneous air breathing, which serves to limit O_2 loss of the gills in aquatic hypoxia.

Rana catesbeiana; hypoxia; amphibian; respiratory regulation; tadpole

THE REGULATION OF RESPIRATORY PROCESSES in all vertebrates depends on information fed back from both mechanoreceptors and chemoreceptors. These receptors, associated with the brain, the respiratory organs themselves, or the arterial vascular system, help to regulate the breathing pattern and also to assure appropriate matching of ventilation and perfusion at the interface between the respiratory medium and the blood. The latter effect is accomplished through reflex adjustments in cardiac output, arterial driving pressure, and even differential distribution of the cardiac output between the systemic and pulmonary circuits in those species with intracardiac shunts (15, 16).

In some respects the control of gas exchange and transport processes attain their highest degree of complexity in those vertebrates that exchange respiratory gases with both air and water. Not only must ventilation and perfusion be regulated in at least two gas exchangers, ventilating different media, but one would presume that O_2 and CO_2 transfer at the gas exchangers must be coordinated and matched to metabolic rate by the activities of the central nervous system.

At least two sensory modalities exist by which such coordination could be achieved. Air-breathing fish appear to possess chemoreceptors located both in the systemic arterial circulation (17) and externally to monitor the O_2 partial pressure (P_{O_2}) of inspired water (6, 10). Also, the activity of aerial gas exchangers (lungs, gas bladders) may be monitored by stretch receptors that supply afferent information on gas volume in the absence of change in the partial pressure of the contained gases (4, 7, 8). In adult bullfrogs, the activity of such lung receptors is also modulated by intrapulmonary partial pressure of CO_2 (P_{CO_2}) (12). The rate and degree of fall in lung P_{CO_2} during inspiration may therefore provide an additional signal to inhibit inspiration in addition to the rate and degree of lung inflation.

We were impressed by the interactions between gill and lung ventilation in larvae of the bullfrog *Rana catesbeiana* in which the lungs, gills, and skin are gas exchange surfaces, and in particular by the marked suppression of gill ventilation which occurs immediately after an air breath in hypoxic water. The functional significance of this respiratory adjustment is to limit the loss of O_2, acquired at the lungs, to the hypoxic water at the gills (22). A similar phenomenon has been described in an air-breathing teleost fish *Anabas* (17). We attempted in this paper to determine whether the interaction in the bullfrog tadpole may be attributed to a changing respiratory drive from putative chemoreceptors after an air breath, or whether it is due to the stimulation of pulmonary mechanoreceptors by the act of lung inflation itself. A study of the reflex effects of lung inflation on gill ventilatory activity in an anuran larva offers a novel opportunity to investigate the reflex interactions possible between two actively ventilated gas exchange organs as well as more specifically providing information on the ontogeny of respiratory control mechanisms in anuran amphibians.

METHODS

Experimental animals. The experiments were performed on 14 *R. catesbeiana* tadpoles (mass = 22.9 ± 2.3 g) at developmental stages XVII–XIX (20). In these stages both internal gills and lungs are functional gas exchangers (2). All animals were collected in western MA

and maintained in filtered water at 25°C for at least 1 wk before the experiments were performed. Tadpoles were maintained on a diet of boiled spinach, available ad libitum.

Surgical preparation. Tadpoles were anesthetized in tricaine methanesulfonate (MS-222) 1:10,000, buffered to pH 7.0. To record gill ventilation we inserted a water-filled cannula (PE-10) through one external narial opening and secured it into the buccal cavity by means of a flange (22). Pressure within the buccal cavity (P_{BC}) was measured by a Narco P1000B pressure transducer. The pressure signal was split and fed to a Narco 7302 Biotachometer to give, in addition, a direct indication of the rate of gill ventilation cycles. Both signals were recorded on a Narco Mark IV four-channel chart recorder writing on rectilinear coordinates. In some preliminary experiments heart rate was also recorded via the electrocardiogram (ECG).

The caudal apex of both lungs was cannulated with air-filled PE-50 tubing (length 20 cm, vol 50 μl) introduced through a 2-mm longitudinal slit in the skin and body wall. The cannulas were tied into the lungs and secured to the body wall. The body wall and skin were subsequently closed separately with interrupted sutures. The open ends of the lung cannulas were plugged during recovery from anesthesia. This preparation allowed either bolus injection of gases into the lungs or a unidirectional ventilation, since the lungs are interconnected via the bronchi caudal to the glottis.

After the surgery tadpoles were allowed to recover in a 1-liter glass chamber containing filtered water, which also served as the experimental chamber. The tadpoles were unrestrained except for the lung and buccal cannulas, which were led above the water surface and supported to reduce catheter drag, which might inhibit spontaneous movements by the tadpole. The P_{O_2} of the water was regulated by passing air, an air-nitrogen mixture, or O_2 through an air stone in the experimental chamber, which was separated from the experimenter by an opaque screen. Water P_{O_2} was measured with an Instrumentation Laboratories (IL) oxygen electrode and either read directly from an IL-113 electrometer or Radiometer PHM-72 electrometer, or displayed on the chart recorder.

Experimental protocol. All experiments were performed at 25 ± 1°C. Three water conditions, hypoxic (P_{O_2} = 80 ± 1 Torr), normoxic (P_{O_2} = 142 ± 1 Torr), or hyperoxic (P_{O_2} = 580 ± 23 Torr) were used. Under the conditions of these experiments, tadpoles only very rarely showed spontaneous air breaths, even in hypoxic water, although the level of hypoxia chosen increased gill ventilation rate and pressure amplitude from the normoxic values (22). Two main experimental protocols were used.

In the first series of experiments the response of gill ventilation to delivery of a humidified gas bolus into the lungs, simulating lung ventilation, was investigated. Air, N_2, or O_2 was used to inflate the lungs of tadpoles in hypoxic, normoxic, or hyperoxic water, yielding nine possible experimental conditions. The gases were delivered via one cannula, the cannula in the other lung being clamped shut. The volume of the bolus of gas was chosen to fully inflate the lungs. Bolus volume was estimated from body mass and data on lung volume for *R. catesbeiana* tadpoles (Burggren and Muralokuma, unpublished observations). Thus, injected gas volumes ranged from 0.2 to 0.4 ml in individual animals. The inflation rate was 0.1 ml/s, resulting in full lung inflation 2–4 s after the start of gas injection. In some trials the effects of rapid lung inflation were investigated, in which case lung inflation was completed in 0.1 s. In no experimental trial was gas observed to leave the lungs via the glottis, although deliberate overinflation beyond estimated lung volume caused the glottis to open and gas bubbles to be released at the mouth. The pressure and rate of gill ventilatory cycles were recorded continuously through the period of experimentation. Aquatic hypoxia, normoxia, and hyperoxia were presented in random order and ventilatory values allowed to stabilize (typically after 60 min) before inflation trials commenced. The inflating gases were presented in random order. After an experimental inflation, gill ventilation frequency and the pressure amplitude of gill ventilation cycles were allowed to stabilize (typically after 3–5 min), a gas volume equal to that delivered was withdrawn via the pulmonary cannula, and the gill ventilatory pressure and rate allowed to return to control values for that aquatic P_{O_2} before a second gas injection was made.

In the second series of experiments we attempted to dissociate the effects on gill ventilation arising from lung inflation per se from those determined by the P_{O_2} of the inflating gas. All of this experimental series was performed in aquatic hypoxia (P_{O_2} = 80 ± 1 Torr) to maximize the observed responses. In experimental periods flow of humidified gas through the lungs was initiated by delivering gas through one pulmonary cannula and allowing it to exit through the patent contralateral cannula. Gas was delivered at rates between 0.54 and 1.29 ml/min, via a Harvard infusion pump. Flow rates were adjusted according to the mass of the animal. The chosen flow rate and outflow resistance of the system were sufficiently low that detectable lung inflation did not occur during the period of through-flow as judged by the absence of buoyancy changes in the animals. After an initial flow period (50 s) the outflow cannula was clamped, but infusion continued and lung inflation was allowed to continue to a calculated 100% of lung volume before being stopped. Again, no bubbles were released from the buccal cavity, indicating that no gas exited via the glottis. After 180 s of maintained inflation, the exhaust cannula was unclamped and ventilation with the experimental gas simultaneously restarted at the original rate for a further 120 s. As in the first series of experiments air, N_2, or O_2 was presented in random order, and in addition between experimental runs the lungs were through-ventilated with humidified air for 120 s to ensure a rapid return to control values of lung gas P_{O_2} and gill ventilation.

Results were analyzed by measuring gill ventilation cycle frequency (f_G) and pressure amplitude (P_{BC}) during the initial control period (C_i); the beginning and end of the initial period of gas flow (F_i, F_f); on the completion of lung inflation (I_{max}); 120 s later at the end of a period of lung inflation (I_f); and during a final control period (C_f).

Statistical analysis. The data were analyzed to provide means and standard errors. Comparison among treat-

ment means was performed with ANOVA and the t test for paired samples where appropriate. Polynomial regressions were plotted with the aid of a BMDP computer program. The fiducial limit of significance was considered to be $P < 0.05$.

RESULTS

Response to P_{O_2}, spontaneous air breaths, and gill ventilation. The relationship between aquatic P_{O_2} and gill ventilation rate and pressure amplitude in stage XVII–XIX tadpoles is illustrated in Fig. 1. These data indicate that there is a significant hypoxic drive to gill ventilation, since both gill ventilation rate and pressure amplitude increase markedly as P_{O_2} falls below normoxic values. However, the onset of spontaneous air breathing in hypoxic water alters this relationship. Gill ventilation cycles following a spontaneous air breath are markedly reduced in amplitude and frequency until the next spontaneous air breath occurs. Figure 2 illustrates this in a stage XIX larva fitted with a narial pressure cannula and ECG leads only. The suppression of gill ventilation following an air breath minimizes the loss of oxygen to the hypoxic water from the blood perfusing branchial capillaries (22). The afferent pathways mediating the effect are the further subject of this investigation.

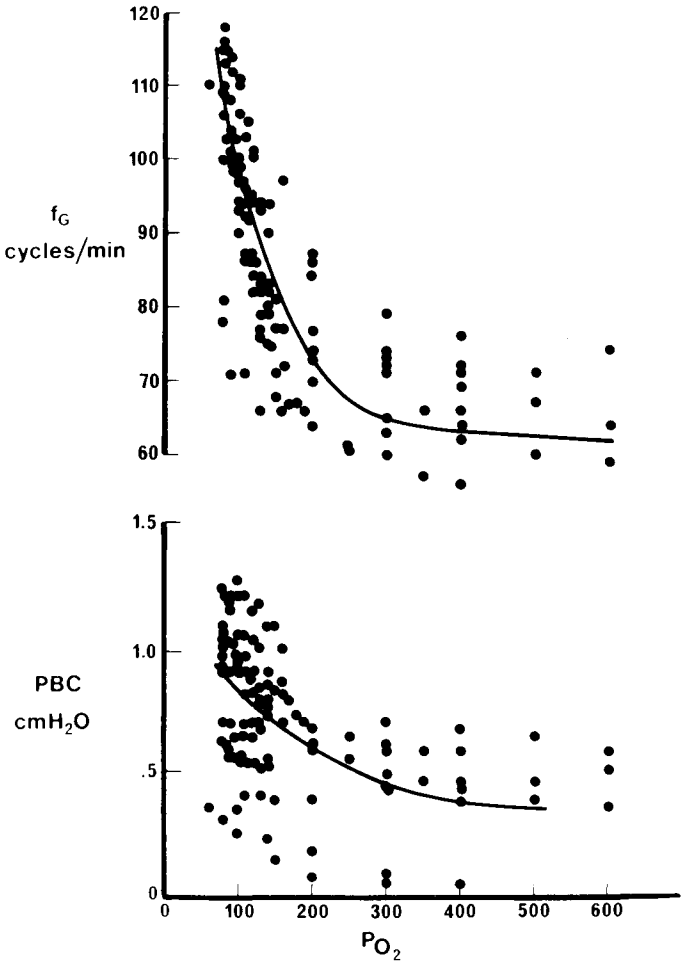

FIG. 1. Relationship between gill ventilation frequency (f_G), pressure amplitude (PBC), and aquatic P_{O_2} in absence of air breathing in 5 *R. catesbeiana* larvae, stages XVII-XIX. Plotted lines represent 3rd degree polynomial regression of data.

Simulated lung inflations. Control gill ventilation frequency (f_G) was significantly higher ($P < 0.02$) in steady-state aquatic hypoxia (P_{O_2} = 80 ± 1 Torr) than in normoxia (P_{O_2} = 142 ± 1 Torr), whereas a significant fall in frequency ($P < 0.05$) occurred in hyperoxic (P_{O_2} = 580 ± 23 Torr) conditions. This was paralleled by changes in the amplitude of buccal pressure oscillations during gill ventilation (PBC), which was significantly lower in normoxia than hypoxia ($P < 0.01$) and significantly lower in hyperoxia than in normoxia ($P < 0.001$) (see Fig. 4).

Lung inflation with any of the three humidified gases resulted in a marked reduction in both f_G and PBC in aquatic hypoxia and normoxia (Figs. 3 and 4), with a maximum effect occurring 20 s after the start of lung inflation. The effect of lung inflation on gill ventilation when the tadpoles were in aquatic hyperoxia was more variable, with f_G being unaffected but PBC nonetheless significantly decreased at 20 s after inflation.

The P_{O_2} of the inflating gas had no statistically significant effect on the reduction in PBC observed at 20 s after lung inflation at any of the three aquatic levels of oxygen. In aquatic normoxia and hyperoxia this held true for the remainder of the period of inflation, however, 65 s after inflation in hypoxic water O_2 inflation resulted in a significantly ($P < 0.05$) lower gill ventilation rate than for air inflation, which in turn was significantly lower ($P < 0.05$) than for N_2 inflation. This difference was maintained until the end of measurement, 145 s after inflation (Fig. 4).

Rapid lung inflation and CO_2 sensitivity. Rapid lung inflations with each gas were carried out in five animals,

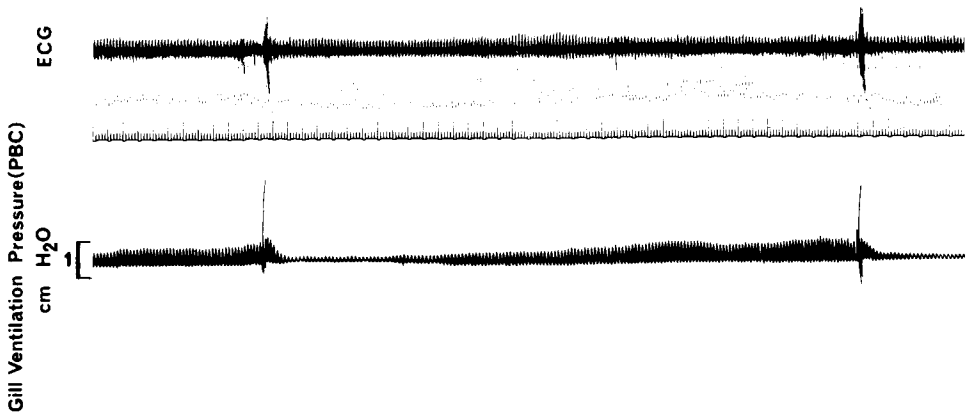

FIG. 2. Suppression of gill ventilation cycles following spontaneous air breaths in a *R. catesbeiana* larva, stage XIX, in hypoxic water (P_{O_2} = 80 Torr). *Upper trace,* electrocardiogram (ECG); *middle,* a 1- and 5-s time marker; *lower,* buccal pressure recorded via a narial cannula.

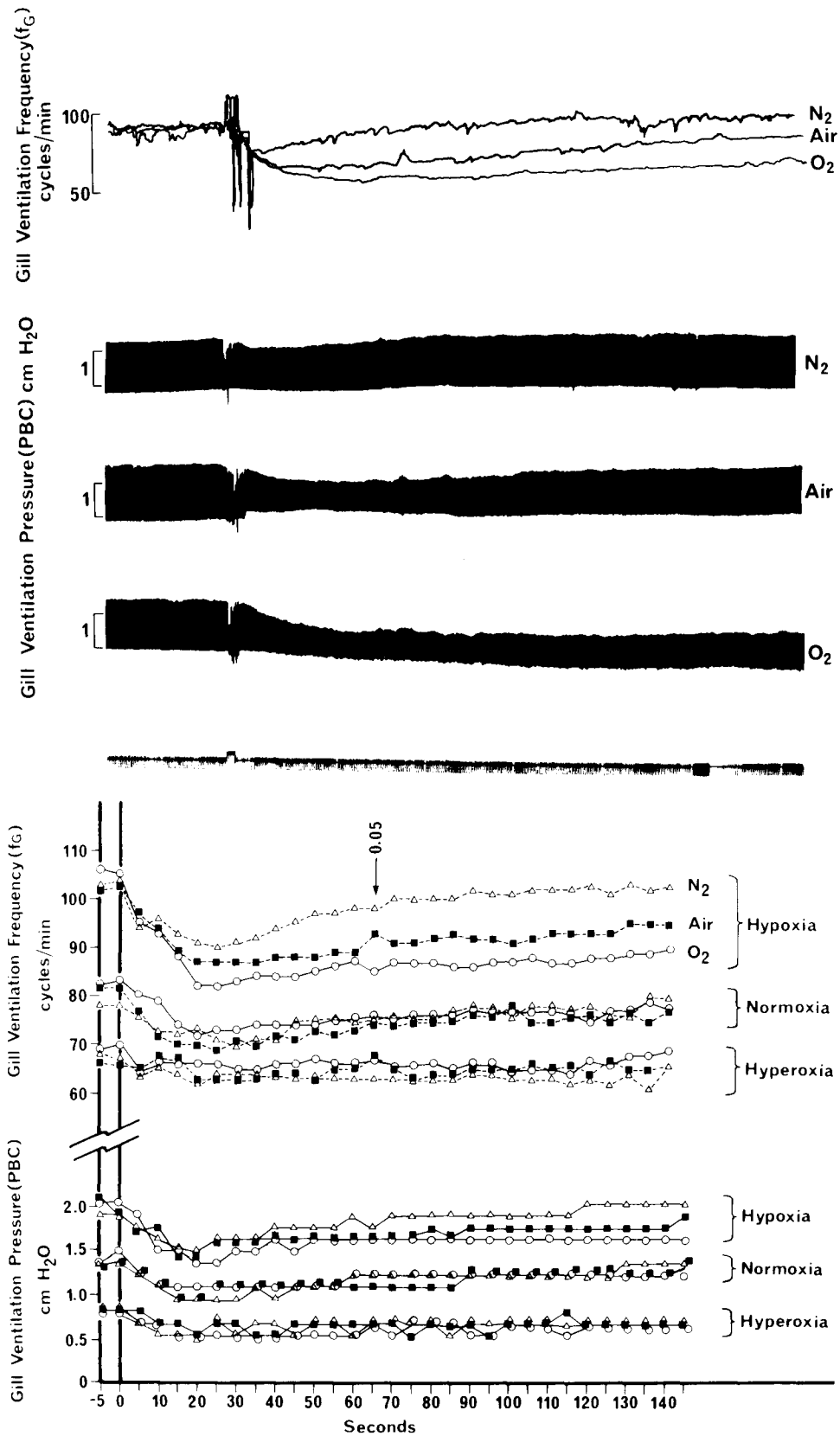

FIG. 3. Effects of lung inflation with N_2, air, and O_2 to an estimated 100% of lung volume in a *R. catesbeiana* larva, stage XX, 35.2 g, in aquatic hypoxia (P_{O_2} = 81 Torr). *Upper traces,* frequency of gill ventilation (f_G); *lower traces,* ventilation cycle pressure amplitude (PBC). *Lowest trace,* a 1-s time marker. Upward deflection indicates lung inflation.

FIG. 4. Gill ventilation cycle frequency (f_G) and buccal pressure amplitude (PBC) for stage XVII–XIX tadpoles in hyperoxic (P_{O_2} = 580 ± 23 Torr), normoxic (P_{O_2} = 142 ± 1 Torr), or hypoxic (P_{O_2} = 80 ± 1 Torr) water. At 0 s lungs were fully inflated via a pulmonary cannula with N_2 (△), air (■), or O_2 (○). n = 7 for hypoxia, 6 for normoxia and hyperoxia. SE not indicated for sake of clarity. See text for details.

in aquatic hypoxia (P_{O_2} = 80 ± 1 Torr), to investigate the time course of the immediate changes in gill ventilation frequency and amplitude in response to lung inflation. Regardless of the P_{O_2} of the inflating gas, rapid (0.1 s) inflations to 100% of lung volume produced reductions in gill ventilation frequency and amplitude within 1 s (Fig. 5*A*), demonstrating that the initial effects of lung inflation must be mediated by the pulmonary stretch receptors rather than changes in lung or blood P_{O_2}.

Adult bullfrogs possess pulmonary stretch receptors

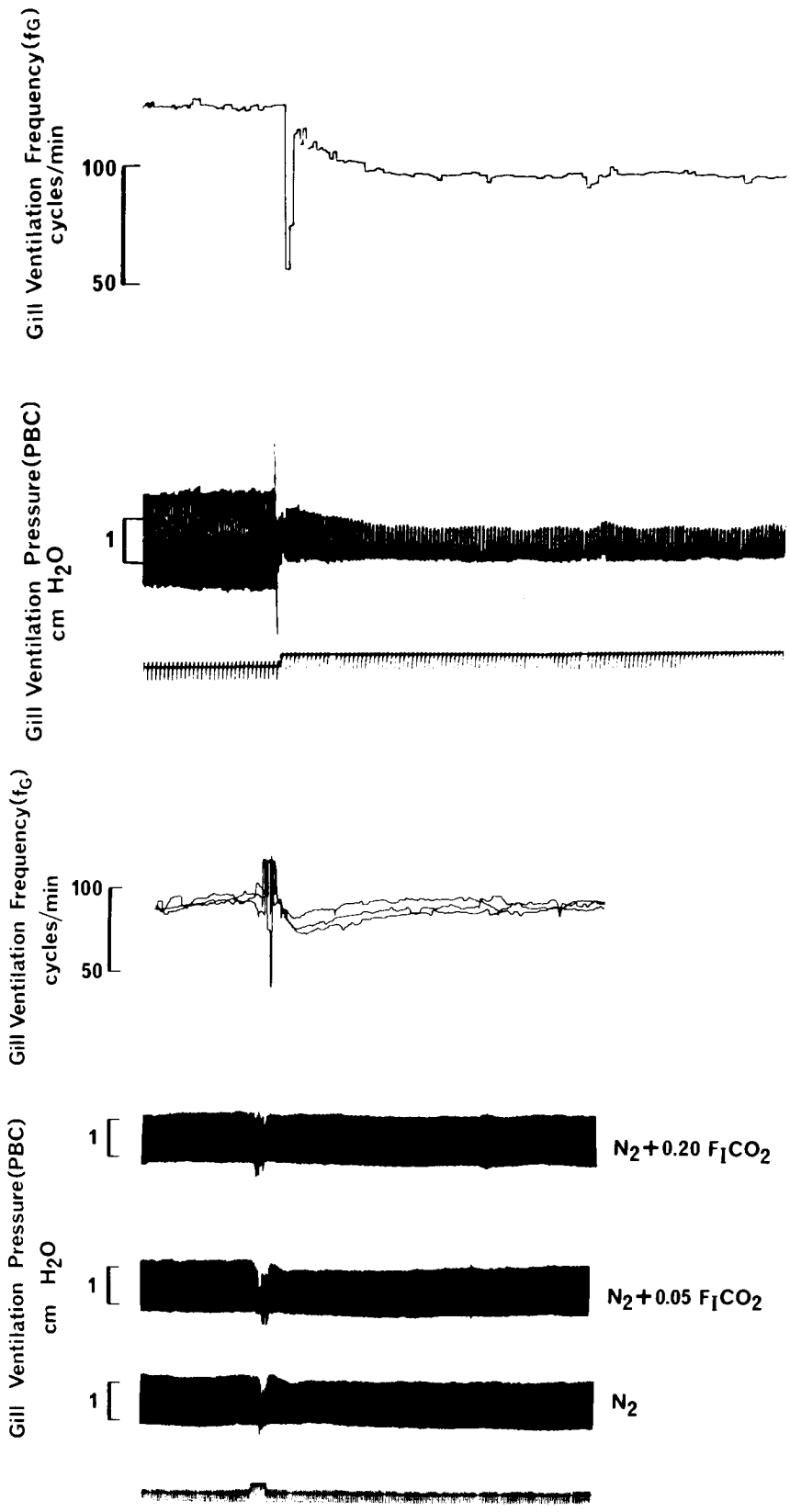

FIG. 5. *A*: effect of rapid lung inflation with 0.4 ml O_2 on gill ventilation frequency and pressure in a *R. catesbeiana* larva, stage XIX, 22.6 g, in aquatic hypoxia (P_{O_2} = 77 Torr). Note immediate reduction in gill ventilation. Rapid inflations with N_2 or air produced similar effects. *Lowest trace* is 1-s time marker. *B*: effects of lung inflation with N_2 and 2 levels of $F_{I_{CO_2}}$ on a *R. catesbeiana* larva, stage XX, 35.2 g, in hypoxic water (P_{O_2} = 81 Torr). An increase in $F_{I_{CO_2}}$ diminishes reduction in gill ventilation observed in response to lung inflation. *Lowest trace* is 1-s time and event marker.

that are CO_2 sensitive and slowly adapting, and whose discharge frequency is decreased within 2 s of CO_2 addition to the ventilating gas (12). Accordingly, we used this characteristic of the receptors to demonstrate the dependence of the initial reduction of gill ventilation frequency and amplitude on stretch receptor input from the lungs on inflation. Figure 5*B* illustrates lung inflation to 100% lung volume in aquatic hypoxia with N_2, N_2 + 0.05 $F_{I_{CO_2}}$, and N_2 + 0.20 $F_{I_{CO_2}}$. The addition of CO_2 to the inflating N_2 reduced the phasic effects of lung inflation on both gill ventilation frequency and amplitude.

Discrimination between effects mediated by P_{O_2} and

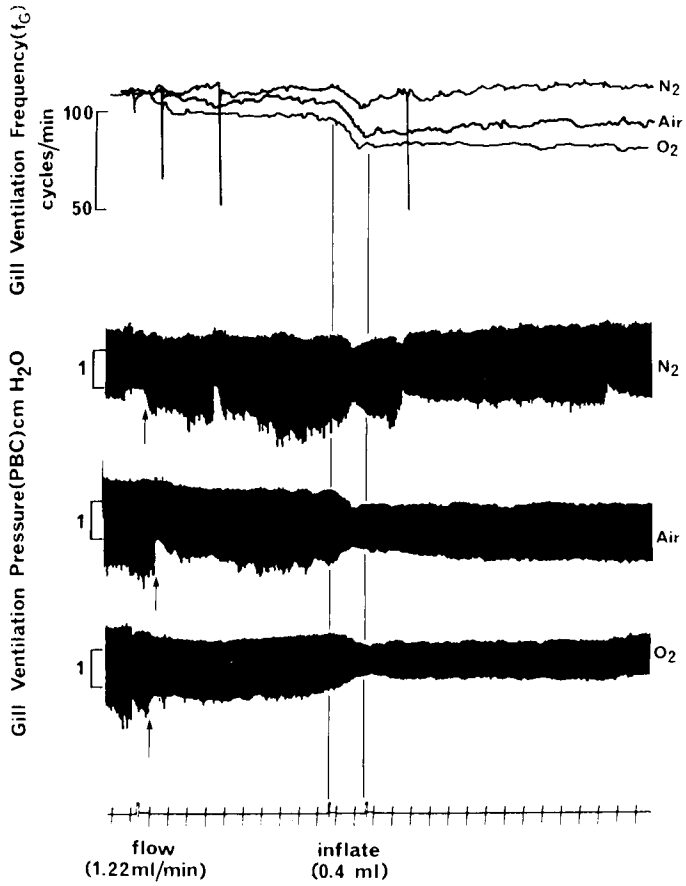

FIG. 6. Effects of through-flow of N_2, air, or O_2 and lung inflation in a *R. catesbeiana* larva, stage XVIII, 24.1 g, in aquatic hypoxia (P_{O_2} = 80 Torr). *Upper traces*, gill ventilation frequencies in response to through-flow of N_2, air, or O_2 have been photographically superimposed. *Arrows*, start of period of through-flow; *vertical lines*, period of lung inflation. After inflation through-flow was resumed at original rate.

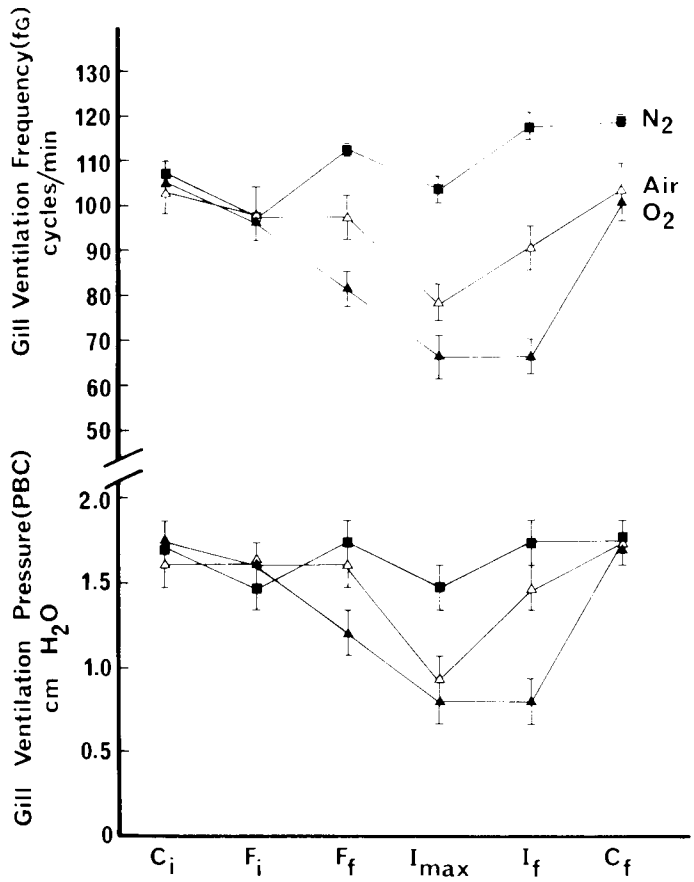

FIG. 7. Effects of through-flow of N_2, air, or O_2 and lung inflation in stage XVII–XIX larvae in aquatic hypoxia (P_{O_2} = 80 ± 1 Torr) on gill ventilation frequency and pressure. C_i, initial control; F_i, 5 s after start of through-flow; F_f, final flow values; I_{max}, maximum effect of lung inflation; I_f, final inflation values; C_f, final control values. Twenty-four observations in 8 larvae. *Vertical lines*, SEM.

lung inflation—through-flow lung ventilation. Gill ventilation rate 5 s after the initiation of through-flow (F_i) was not significantly different from the initial control values (C_i) for ventilation with N_2, air, or O_2 (Figs. 6 and 7). By the end of the 120-s period of flow (F_f) however, f_G for N_2 flow was significantly ($P < 0.05$) greater than for airflow, which in turn was significantly higher than for O_2 flow ($P < 0.02$).

Once flow was established, lung inflation to 100% of estimated lung volume (I_{max}) produced a significant ($P < 0.05$) fall in gill ventilation frequency, independent of the P_{O_2} of the flowing gas. This effect of lung inflation on gill ventilation frequency in the absence of P_{O_2} change was maintained to the end of the period of lung inflation (120 s) if the inflating gas was O_2, whereas gill ventilation frequency increased from that at I_{max} for air and N_2 ventilations. The f_G measured during a final control period (C_f) 5 min after the end of inflation were not significantly different from those during C_i for air and O_2 through-flow but significantly higher for N_2 through-flow ($P < 0.02$). The effects of the protocol on PBC demonstrated the same trends as those on f_G (Fig. 7) although at a lower level of significance. By the end of the initial period of gas flow (F_i) the pressure amplitudes attained were not significantly different from the initial control values (C_i). Lung inflation reduced PBC in each gas treatment, but only on air inflation was this effect statistically significant ($P < 0.01$). At I_{max} the PBC values for N_2 and O_2 inflation were significantly different ($P < 0.001$). By the end of the period of lung inflation there was a significant ($P < 0.01$) increase in PBC from the I_{max} value for air inflation, but changes for the other gases were not significant. Final control values (C_f) were not significantly different from the initial control values (C_i).

DISCUSSION

In bullfrog tadpoles in hypoxic water the gill ventilation cycles following a spontaneous air breath are reduced in frequency and pressure amplitude, which serves to reduce the flow rate of hypoxic water over the gills (22). Teleologically, the adaptive significance of such a modulation of gill ventilation by lung inflation in the bullfrog tadpole is that it limits the loss of blood-borne oxygen, acquired by lung ventilation, to the water at the gills. This could occur in conditions in which the P_{O_2} of venous blood perfusing the aquatic gas exchange organ is higher than the P_{O_2} of inspired water. In the absence of such a mechanism a tadpole in severely hypoxic water would in effect act as a pump transferring O_2 from air to water at the gills (1). It should be noted that there is a significant amount of cutaneous O_2 exchange in normoxic tadpoles (2). The skin is perfused in series with the gills but lies downstream from them. The local partial pressure gra-

dients between skin and water may also be reversed in hypoxic water after an air breath. The potential therefore exists for O_2 loss at the skin as well as the gills. The possibility that cutaneous perfusion is reduced under these circumstances remains to be explored.

Our results for larval bullfrogs indicate that lung inflation in conditions of aquatic hypoxia modulates the activity of the central respiratory oscillator driving gill ventilation by at least two discrete afferent pathways, subserving pulmonary stretch receptors and oxygen-sensitive chemoreceptors.

1) In hypoxic water experimental lung inflation regardless of the P_{O_2} of the inflating gas, caused a reduction in the frequency and pressure amplitude of the gill ventilation cycles that followed, with a maximal response after some 20 s. In response to rapid inflation, the onset of the response occurred more rapidly (<1 s), suggesting a rate-sensitive system. These data together indicate a neural response stimulated by the increase in pulmonary pressure, or volume, but not by changes in lung gas or blood P_{O_2}. Information on the extent of lung inflation, mediated by pulmonary stretch receptors, causes profound ventilatory changes, independently of the chemical composition of the inflating gas, in air-breathing fish, reptiles, and amphibians (4, 7, 8, 21). In the bullfrog tadpole, in which comparatively little CO_2 enters the lungs between breaths [pulmonary R = 0.2 in aquatic normoxia (2)] lung volume information may also serve indirectly as a detector of the volume of the remaining pulmonary O_2 store after an air breath, perhaps providing one afferent channel by which the next air breath is triggered.

Slowly adapting pulmonary stretch receptors capable of mediating such a response have been neurophysiologically explored in adult anuran amphibians (12, 19). Our view that such receptors are involved in the inhibition of larval gill ventilation following lung inflation appears to be strengthened by the evidence that high $F_{I_{CO_2}}$ in the inflating gas reduces the gill ventilation response to lung inflation in the bullfrog tadpole, for Kuhlmann and Fedde (12) demonstrated that in the adult bullfrog the discharge of such receptors is decreased by lung inflation with air containing 5–10% CO_2. Toews (21) noted that in adult *Amphiuma* the onset of the next spontaneous air breath occurred more rapidly if a mixture of air + 20% CO_2 was injected into the lungs rather than air-CO_2 mixtures up to 15%, perhaps again implying the involvement of such receptors in respiratory control.

2) In aquatic hypoxia there is a significant difference in the reduction in gill ventilation mediated by N_2, air, or O_2 by 65 s after experimental lung inflation, with O_2 inflation being most effective in reducing the amplitude and frequency of the gill ventilation cycles that follow. This suggests that O_2 acquired by the lungs in a natural air breath serves to alleviate partially a tonic hypoxic drive on gill ventilation. The site of the putative O_2-sensitive elements in the bullfrog tadpole is currently unknown, although peripheral chemoreceptor sites, capable of elevating ventilation in response to hypoxia or cyanide injection, are associated with the carotid labyrinth in adult anurans (5, 13, 18). Many aquatic vertebrates possess both interoceptive and exteroceptive O_2-sensitive chemoreceptors, their locations including the pseudobranch, first gill arch, dorsal aorta, and brain (14). Our experimental evidence from the bullfrog tadpole appears to point to an interoreceptor responding to P_{O_2}, which does not differentiate between an O_2 signal derived from ventilating water or gas, because increase in the P_{O_2} of either medium ultimately alleviates the hypoxic drive to gill ventilation.

It is interesting to note that elevation of aquatic P_{O_2} above the normoxic value resulted in a marked reduction in gill ventilation [see also West and Burggren (22)]. The threshold for a ventilatory response to hypoxia was above 200 Torr in our animals. The presence of a hypoxic ventilatory drive at normoxic aquatic P_{O_2} is the rule in obligate water-breathing fish (3), but the situation is not so clear-cut among air-breathing fish, for example, aquatic hypoxia decreases gill ventilation in *Amia* (9), whereas the threshold for a ventilatory response to hypoxia is about 100 Torr in *Neoceratodus* (11).

Through-flow ventilation of the lungs has allowed experimental dissociation of the effects mediated by pulmonary stretch and the P_{O_2} of the inflating gas on gill ventilation. Through-flow of N_2 led to an increase in gill ventilation frequency and amplitude and through-flow of O_2 a fall, results to be expected on the basis of the modulation of hypoxic drive. However, lung inflation per se, irrespective of the P_{O_2} of the ventilating gas stream and the associated changes in gill ventilation, always resulted in a reduction of gill ventilation from that observed in through-flow. This indicates that the change in lung volume on inflation acts as a stimulus independent of the P_{O_2} of lung gas.

Interestingly, bolus lung inflation with all gases had less of an effect on gill ventilation in aquatic normoxia and hyperoxia than hypoxia. Therefore, the input from pulmonary stretch receptors is most effective in modulating gill ventilation in aquatic hypoxia. This may be an indication of a central interaction between stretch receptor and chemoreceptor pathways sensitive to aquatic P_{O_2}, which was not apparent in the through-flow experiments.

To our knowledge, no comparable study investigating the pathways by which gill ventilation is modulated by lung inflation has been performed on amphibian larvae, although the presence in larval anurans of two distinct gas exchangers, whose activities reflexly interact, must surely impose constraints on the design of the neural mechanisms controlling ventilation in adult anuran amphibians.

At the time this work was performed the authors were supported by grants from the National Science and Engineering Research Council (Canada) and Medical Research Council (Canada) awarded N. H. West and the National Science Foundation awarded W. W. Burggren.

Received 17 August 1982; accepted in final form 4 January 1983.

REFERENCES

1. BURGGREN, W. W. Bimodal gas exchange during variation in environmental oxygen and carbon dioxide in the air breathing fish, *Trichogaster trichopterus*. *J. Exp. Biol.* 82: 197–213, 1979.
2. BURGGREN, W. W., AND N. H. WEST. Changing respiratory importance of gills, lungs and skin during metamorphosis in the bullfrog *Rana catesbeiana*. *Respir. Physiol.* 47: 151–164, 1982.
3. DEJOURS, P. *Principles of Comparative Respiratory Physiology*. Amsterdam: Elsevier/North-Holland, 1981.

4. EMILIO, M. G., AND G. SHELTON. Factors affecting blood flow to the lungs in the amphibian, *Xenopus laevis*. *J. Exp. Biol.* 56: 67–77, 1972.
5. ISHII, K., K. HONDA, AND K. ISHII. The function of the carotid labyrinth in the toad. *Tohoku J. Exp. Med.* 88: 103–106, 1966.
6. JOHANSEN, K. Chemoreception in respiratory control of lungfish, *Neoceratodus* (Abstract). *Federation Proc.* 25: 389, 1966.
7. JOHANSEN, K. Air breathing in fishes. In: *Fish Physiology,* edited by W. S. Hoar and D. J. Randall. New York: Academic, 1970, vol. 4, p. 361–411.
8. JOHANSEN, K., W. BURGGREN, AND M. GLASS. Pulmonary stretch receptors influence pulmonary blood flows and heart rate in the turtle, *Pseudemys scripta*. *Comp. Biochem. Physiol. A* 58: 185–191, 1977.
9. JOHANSEN, K., D. HANSON, AND C. LENFANT. Respiration in a primitive air breather, *Amia calva*. *Respir. Physiol.* 9: 162–174, 1970.
10. JOHANSEN, K., AND C. LENFANT. Respiration in the African lungfish *Protopterus aethiopicus*. II. Control of breathing. *J. Exp. Biol.* 49: 453–468, 1968.
11. JOHANSEN, K., C. LENFANT, AND G. C. GRIGG. Respiratory control in the lungfish, *Neoceratodus forsteri* (Krefft). *Comp. Biochem. Physiol.* 20: 835–854, 1967.
12. KUHLMANN, W. D., AND M. R. FEDDE. Intrapulmonary receptors in the bullfrog: sensitivity to CO_2. *J. Comp. Physiol.* 132: 69–75, 1979.
13. LILLO, R. S. Localization of receptors which may cause diving bradycardia in bullfrogs. *Can. J. Zool.* 58: 931–936, 1980.
14. RANDALL, D. J., W. W. BURGGREN, A. P. FARRELL, AND M. S. HASWELL. *The Evolution of Air Breathing in Vertebrates*. Cambridge, UK: Cambridge Univ. Press, 1981.
15. SHELTON, G. The effect of lung ventilation on blood flow to the lungs and body of the amphibian, *Xenopus laevis*. *Respir. Physiol.* 9: 183–196, 1970.
16. SHELTON, G., AND W. W. BURGGREN. Cardiovascular dynamics of the chelonia during apnoea and lung ventilation. *J. Exp. Biol.* 64: 323–343, 1976.
17. SINGH, B. N., AND G. M. HUGHES. Cardiac and respiratory responses in the climbing perch *Anabas testudineus*. *J. Comp. Physiol.* 84: 205–226, 1973.
18. SMYTH, D. H. The central and reflex control of respiration in the frog. *J. Physiol. London* 95: 305–327, 1939.
19. TAGLIETTI, V., AND C. CASELLA. Stretch receptor stimulation in frog's lungs. *Pfluegers Arch.* 292: 297–308, 1966.
20. TAYLOR, A. C., AND J. KOLLROS. Stages in the normal development of *Rana pipiens* larvae. *Anat. Rec.* 94: 7–24, 1946.
21. TOEWS, D. P. Factors affecting the onset and termination of respiration in the salamander, *Amphiuma tridactylum*. *Can. J. Zool.* 49: 1231–1237, 1971.
22. WEST, N. H., AND W. W. BURGGREN. Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole. *Respir. Physiol.* 47: 165–176, 1982.