Ontogeny of heart rate regulation in the bullfrog, *Rana catesbeiana*

WARREN BURGGREN AND MICHAEL DOYLE
Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01003-0027

BURGGREN, WARREN, AND MICHAEL DOYLE. *Ontogeny of heart rate regulation in the bullfrog, Rana catesbeiana.* Am. J. Physiol. 251 (Regulatory Integrative Comp. Physiol. 20): R231–R239, 1986.—Heart rate (f_H) at 20–23°C was recorded in six different developmental stages of the bullfrog, *Rana catesbeiana* (n = 104, body mass 40 mg to 90 g), at rest after normoxic acclimation, during acute changes in O_2 availability, and after brief but intense activity. The effects of cholinergic blockade and combined cholinergic and β-adrenergic blockade on the response to this experimental protocol were also assessed. Mild tonic vagal inhibition of f_H was evident during larval development but disappeared after metamorphosis. There was no tonic sympathetic stimulation of f_H at rest in any developmental stage. Intense activity produced a tachycardia in all developmental stages but newly hatched larvae. In adults, tachycardia during activity resulted from β-adrenergic stimulation but in larvae may have resulted from the direct effects of increased venous return stretching the cardiac pacemaker. Neither acute hypoxia or hyperoxia affected f_H in any developmental stage, with the single exception of a severe depression of f_H occurring at a P_{O_2} of 30 Torr in newly hatched larvae. These results indicate that, although the heart of the newly hatched larvae is essentially devoid of regulation, cholinergic and β-adrenergic mechanisms for reflex regulation of f_H appear early in larval development. These mechanisms, although most fully expressed in the adult bullfrog, are essentially intact before metamorphosis of the larva.

vagal tone; metamorphosis; amphibian; development; atropine; propranolol; baroreceptors; hypoxia; exercise

EMBRYONIC ORIGINS of cardiovascular regulation are poorly understood in vertebrates. Even in mammals, quantitative information about the fetal circulation has been acquired almost exclusively from fetuses in the last one-half to one-third of gestation, by which time most adult cardioregulatory mechanisms are already largely or entirely functional (16). Embryos and larvae of nonmammalian vertebrates have been used as systems for studying the ontogeny of cardiorespiratory regulation (see Ref. 12), but the embryonic origins of cardiovascular reflexes in these animals remain particularly obscure.

Several characteristics of the developing amphibian larva provide unexploited opportunities for the study of the ontogeny of vertebrate cardiorespiratory regulation. For example, most amphibian larvae hatch from eggs into free-living entities at a very immature morphological and physiological stage of development compared with reptiles, birds, or mammals (5). Thus larvae are readily accessible for physiological experimentation at early stages of development. This precocious entrance into the external environment also results in the amphibian larva being completely exposed throughout its development to the same highly variable habitat as the adult, unlike the comparatively benign and protected uterine environment enjoyed by the mammalian fetus. Amphibian larvae therefore must survive environmentally induced stresses, even though some physiological regulatory mechanisms may not yet be fully or even partially functional. Not only does this raise interesting physiological questions, but it also facilitates investigation. For example, hypoxic or hypercapnic stresses can be induced much more easily and directly in the amphibian larva than in the bird embryo or mammalian fetus, both of which are much more buffered from environmental changes.

The amphibian larva thus presents a compelling system for the study of the ontogeny of vertebrate cardiovascular regulation. A substantial body of information on regulation of heart rate, blood pressure, and regional blood flow exists for adult amphibians (2, 19, 26, 27), but the literature is bereft even of speculation on the developmental origins of cardiovascular regulation in amphibians. There are many crucial unanswered questions of wide relevance to developmental biology and physiology. At what developmental stage does the amphibian heart become functionally innervated, and does this coincide with the onset of hormonal regulation? At what stage in ontogeny does effective matching of ventilation to perfusion begin in the respiratory organs? When do reflexes modulating cardiovascular function in response to environmental change develop, and how do they change with metamorphosis?

To begin to answer these questions, this study tests the following hypotheses: *1*) the heart of the bullfrog larva at hatching is essentially devoid of regulation; *2*) the bullfrog larva develops the capacity for cardiac regulation well before metamorphosis; and *3*) larval and adult cardiac regulatory mechanisms may differ as a consequence of different modes and media for respiration.

MATERIALS AND METHODS

Animals

Experiments were performed on larval stages, on postmetamorphic adults of small mass, and on fully grown

adults of the bullfrog, *Rana catesbeiana*. With the exception of the fully grown adults, which were acquired from commercial suppliers, all animals were captured in Hampshire County, MA. The animals were maintained in the laboratory at 20–23°C on a 12:12 photoperiod for at least 1 wk before experimentation and were regularly fed either spinach (larvae) or flies and maggots (adults). Experiments were performed in the winter of 1983 and the spring and summer of 1984.

Experiments were performed on animals from six different developmental ranges, classified according to the scheme of Taylor and Kollros (22), with salient features as follows. *Stage I*: 1- to 3-day-old larvae; buccal movements evident but gills external and nonventilated; *stage IV–VII*: internal and actively ventilated gills; lungs present but not ventilated; very small limb buds; *stage X–XIV*: lung ventilation begins; phalanges evident on rear legs; *stage XVI–XIX*: rear legs well developed; front legs well developed but still internal; *stage XX–XIV*: gape of mouth widens; internal gills degenerate; tail reabsorbed; adults: metamorphosis complete; gas exchange via lungs and skin; large difference in body mass depending on age.

Heart Rate Measurement

Because of the intentionally large range in body mass of the experimental animals, several different experimental techniques were used. The heart of newly hatched larvae [mean mass 40 ± 9 (SD) mg] could be viewed directly through the translucent skin of the ventral body wall. To facilitate viewing, newly hatched larvae were placed in individual glass tubes (3 mm ID) and confined to a region 3 cm in length by placing a barrier of cotton fibers behind and in front of the animal. Each of the 15 larvae examined could voluntarily move several body lengths forward or backward but could not turn around within the tube. A constant gentle flow of water with a specific gas partial pressure was maintained through the tubes. Larvae tolerated this apparatus well, and although spontaneous locomotor movements were observed, these were of similar frequency and duration as those of larvae kept unconfined in large aquariums. A mirror was arranged under the array of glass tubes, and by using a dissecting microscope at ×30 magnification, the frequency of heart beat of each larva could easily be determined.

For older larvae and adults, heart rate (f_H) was recorded via implanted electrocardiogram (ECG) electrodes. Animals were first anesthetized, either by immersion in MS 222 (1:10,000, buffered to pH 7.0) for larvae or by injection of urethan (3 mg·kg body mass^{-1}) into the dorsal lymph sac of adults. ECG electrodes consisted of fine insulated copper wires (no. 45 gauge for *stage IV–VII*, no. 40 gauge for other larval stages, no. 36 for adults) bared for 1 mm at their tip. A pair of electrode wires were inserted subcutaneously via needles at points ~2 mm apart directly over the heart and were secured with fine sutures to the integument (see Refs. 24 and 25 for details). A third wire serving as a ground was inserted and secured caudally. In most animals an additional pair of electrode wires was inserted on either side of the mouth (larvae) or chest (adults) to allow recording of ventilatory activity. All electrode wires were gathered together, led over the skin to the dorsal surface of the animal, and secured to the skin with an additional suture.

Animals fitted with electrodes were placed in one of four separate compartments of a small water-filled aquarium (16.5 × 30.5 × 20.5 cm). The aquarium was fitted with a gas-tight lid providing a 4-cm gas space above the water level. The electrodes from each animal were led out through ports in the lid and tethered to an overhead support to keep the electrode cable from interfering with or restricting movements of the animal. Both larvae and adults apparently tolerated this procedure very well, as evidenced by similar behaviors and rates of gill and lung ventilation in completely unrestricted animals without electrode wires. The electrodes for recording of ECG and gill and/or lung ventilation frequency were connected to biopotential preamplifiers and instantaneous ratemeters of a Narco MK-IV rectilinear recording system.

Tap water at 20.0 ± 0.7°C was pumped through a gas exchange column and then to each of the four compartments of the aquarium. P_{O_2} of the water was adjusted by using flowmeters to regulate the flow of an O_2-N_2 gas mixture through the gas exchange column. The P_{O_2} of water in the four experimental compartments, as well as water temperature, was continuously recorded using a Yellow Springs Instruments model 56 O_2 monitor. The P_{O_2} of the gas space above the water was maintained within 5 Torr of the P_{O_2} of the underlying water by directing the output from an appropriately set Wösthoff gas mixing pump into the gas space.

Drug Treatment

Stock solutions (10^{-2} M) of atropine sulfate (a cholinergic antagonist) and propranolol hydrochloride (a β-adrenergic antagonist) in amphibian saline were prepared for later dilution. Newly hatched larvae (*stage I*, mass ~40 mg) were too small for drug injection, but in all older larval stages, drug solutions were administered by injection through the ventral body wall into the peritoneal cavity. In adults, solutions were injected into the dorsal lymph sac between the eyes. Injections could be completed within ~10 s of first disturbance of the animal. Both atropine and propranolol were administered in doses of 1 mg·kg body mass^{-1}. Preliminary experiments involving injection of various combinations of atropine (ATR) and acetylcholine or of norepinephrine and propranolol (PRO) were performed to determine effective doses and onset times for antagonist action. Both ATR and PRO produced pharmacological blockade of the heart within 30 min of injection into the peritoneal cavity.

Experimental Protocol

All animals were given at least 12 h under normoxic conditions to recover after electrode implantation. After

this recovery period, recordings of ECG and ventilation were made under normoxic conditions to serve as the first of several control data sets. Ambient P_{O_2} was then changed progressively over a 30-min period from 150 Torr down to 90 Torr. f_H almost always stabilized within 10–15 min at this new P_{O_2} level. After recording stabilized f_H at 90 Torr, the water P_{O_2} was reduced to 60 Torr over 30 min, and the stabilized f_H at this new P_{O_2} was recorded. This procedure was repeated once again to P_{O_2} of 30 Torr, after which P_{O_2} was raised to >500 Torr for a final measurement of f_H in this first experimental series. After exposure to hyperoxia, animals were returned to normoxia (~150 Torr) for 1–2 h. f_H at the end of this normoxic recovery period was not significantly different ($P > 0.1$, see *Statistical Analysis*) from the resting control f_H recorded before hypoxic-hyperoxic treatment.

After this second normoxic acclimation period, each animal was mechanically stimulated to produce intense sustained locomotor movements for 1-min duration. Recordings of f_H in response to activity were then begun immediately and continued for 5 min. Intense activity, even of brief duration, can have long-lasting effects on metabolic rate and lactate accumulation in the larvae of *R. catesbeiana* (e.g., Ref. 17). Thus it is important in interpreting the present experiments to emphasize that f_H invariably returned to values not significantly different from resting preactivity rates well within the 1-h recovery period and usually within 20 min. Consequently, each animal was used for subsequent experimental treatments with the assumption that previous treatment history, i.e., activity, did not affect f_H recorded after the recovery period.

After completion of the above protocol, including variations in ambient P_{O_2} and activity interspersed with recovery periods, ATR was injected (except for *stage I* larvae), and 60 min were allowed for cholinergic blockade to develop and for f_H to stabilize. Preliminary experiments indicated that cholinergic blockade persisted for at least 8 h after injection of ATR. Sham injections with saline revealed that a 60-min postinjection acclimation period was sufficiently long for f_H to return to a resting level after the brief handling for injection. Any effect persisting at the end of this recovery period was thus due to the drug treatment. f_H in cholinergically blocked resting animals was then recorded during normoxia, hypoxia ($P_{O_2} = 30$ Torr), hyperoxia ($P_{O_2} > 500$ Torr), a normoxic recovery period, and during brief intense activity, according to the protocol outlined above. Once again, the resting normoxic f_H of atropinized animals was not significantly different before and after recovery from the protocol.

β-Adrenergic blockade was then produced in these cholinergically blocked animals by injection of PRO, and the experimental sequence described above for atropinized animals was repeated again.

Effects of Venous Return on Heart Rate

Experiments were carried out on *stage XVI–XIX* larvae and small postmetamorphic adults (all of similar mass, see Table 1) to determine if there were chronotropic cardiac effects produced strictly by changes in venous return. The central nervous system of larvae or adults was destroyed by a blow to the head or by pithing, respectively. The hepatic portal vein was immediately exposed and cannulated occlusively in a downstream direction with a PE-20 cannula attached to an elevated reservoir containing aerated amphibian saline. The truncus arteriosus was entirely severed 1 mm distal to the heart, but the heart was left intact. ECG was recorded via a pair of no. 45 gauge copper electrode wires placed on either side of the ventricle. The resulting preparation was essentially an intact heart receiving all venous return from the reservoir of saline and ejecting against near-zero peripheral resistance. By eliminating ejection into the arterial circulation, any arterial baroreceptor influence on f_H that remained after central nervous system destruction was removed as a variable. Any change in f_H in response to changing infusion of saline via the hepatic portal vein would be due either to stretch receptors located in the walls of the veins or the heart or to direct effects of stretch on the pacemaker cells of the sinus venosus.

Saline was infused for 10-s periods separated by at least 2 min, using infusion rates of ~300 μl saline·g body mass^{-1}·min^{-1}. Infusion caused a distension of the central veins and heart that, on visual inspection, appeared the same as that observed at end diastole in the intact circulation. After control responses of f_H to infusions were recorded, 500 μl of 10^{-3} M ATR were pipetted directly onto the ventral surface of the heart, and 5 min were allowed for cholinergic blockade to develop. Saline infusions were repeated after the onset of cholinergic blockade. Finally, 500 μl of 10^{-3} M PRO were applied to the heart, and a third series of saline infusions was performed.

Statistical Analysis

Data are presented as means ± SE. Treatment effects (i.e., variations in ambient P_{O_2}, locomotor activity, and drug applications) were tested for significance with one-way analysis of variance (ANOVA). Each developmental group was considered independently. Where ANOVA revealed significant treatment effects, differences between individual means within a developmental group were subsequently assessed for significance with the *T* method. This procedure allows for unplanned multiple comparisons among pairs of means based on equal sample size and generates a minimum significant difference by which two means must differ (28). A fiducial limit of 0.05 was adopted for all analyses.

RESULTS

Heart Rate at Rest

Influence of development stage. Mean values of resting f_H (20°C, normoxia) for six different developmental stages of the bullfrog are indicated in Fig. 1. Figure 2 shows representative tracings of ECG and f_H in five

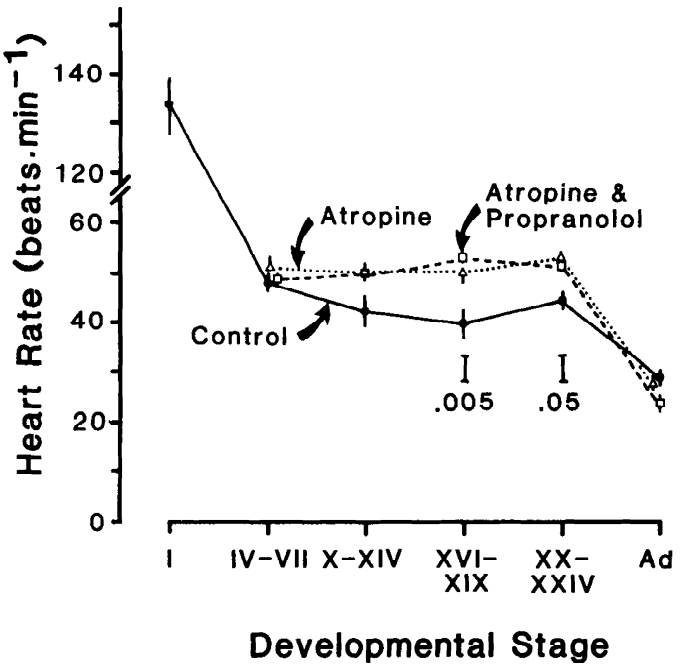

FIG. 1. Heart rate in unrestrained *Rana catesbeiana* at rest in normoxia (20°C), as a function of developmental stage before and after cholinergic (atropine) and combined cholinergic and β-adrenergic blockade (atropine plus propranolol). Values are means ± SE. Number of animals used is 12, 10, 12, 12, 13, and 15 from *stage I* through adult, respectively. If ANOVA revealed significant differences between control, atropine, and atropine + propranolol treatments within a single developmental group, then level of significance is indicated below mean values for that group. Also shown for that group is minimum significant difference for unplanned multiple comparisons, by which 2 mean values within any given developmental stage must differ if they are significantly different. See text for further details.

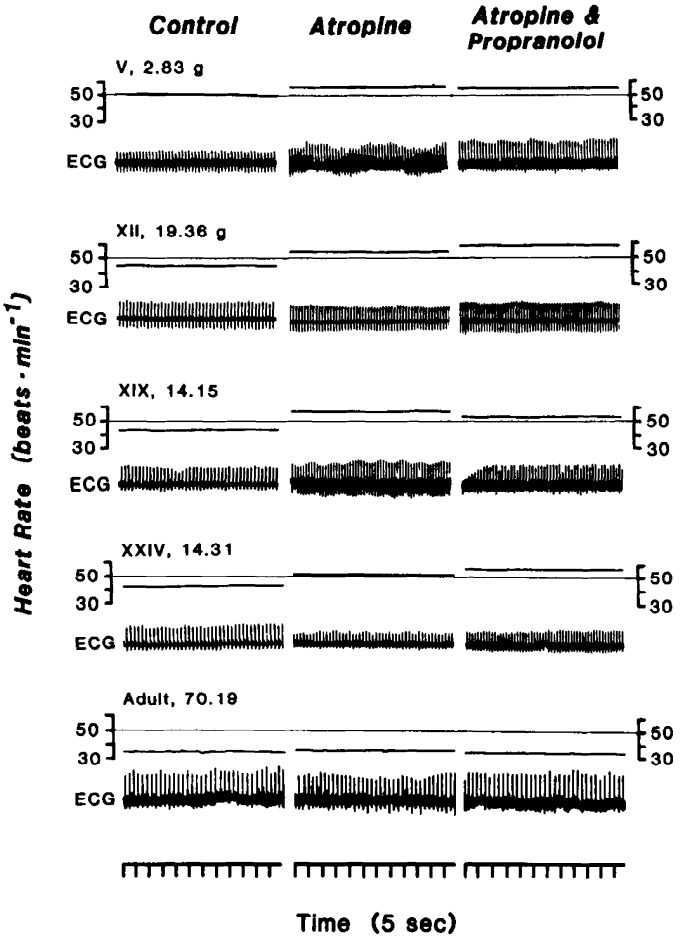

FIG. 2. Representative records of electrocardiogram (ECG) and instantaneous heart rate from unrestrained resting *Rana catesbeiana* before and after cholinergic and combined cholinergic and β-adrenergic blockade in normoxia. Traces are shown for 5 different developmental stages. Each time mark represents 5-s interval.

resting normoxic individuals representing all but the youngest development stage examined. Immediately after hatching, f_H at 25°C was ~135 beats·min^{-1} (Fig. 1). However, development to *stage IV* was accompanied by a precipitous decline in resting f_H to ~40–50 beats·min^{-1}. This lower f_H typified the rest of larval development through forelimb emergence and significant tail regeneration (*stages XX–XXIV*). On completion of metamorphosis, f_H showed a final decline to the adult rate of ~30 beats·min^{-1} (Figs. 1 and 2).

Spontaneous changes in heart rate. The presence of spontaneous transient changes in resting f_H gives some indication of an animal's ability to regulate cardiac function. In the present study, resting f_H in individual larvae immediately after hatching varied <3–5%, even during several hours of observation. Phase coupling between heart and buccal pump was never observed in these early larval stages. In all older larval stages, spontaneous changes in f_H occasionally were observed in resting stationary animals. However, f_H showed no change during either spontaneous cessation of gill ventilation lasting several minutes in larvae of any stage (Fig. 3) or by an air breath in more advanced larvae (Fig. 4*B*). Only if an air breath was accompanied by considerable locomotor movement before or after surfacing to breathe would f_H change, and these effects were more likely associated with activity rather than respiration per se (see below). As in newly hatched larvae, there was no evidence of cardiorespiratory synchrony in resting larvae of later developmental stages (Fig. 3).

Unlike larvae, adults frequently showed a tachycardia lasting for 30–90 s immediately after an air breath (Fig. 4*A*). The increases above resting apneic f_H were not large (10–20%) but were considerably greater than the usual spontaneous variations in f_H occurring during apnea.

Effects of atropine and propranolol. The effects of ATR and ATR + PRO on resting f_H in larvae and adult bullfrogs were assessed to determine the extent of cholinergic and β-adrenergic tonic cardiac stimulation at rest. In early developmental stages (*IV–VII* and *X–XIV*), no drug combination induced any significant effect on resting f_H (Figs. 1 and 2). However, in later developmental stages (*XVI–XIX* and *XX–XXIV*), ATR alone induced a significant increase of ~10 beats·min^{-1} (~25%) in resting f_H. Mean f_H after ATR + PRO was not significantly different from mean f_H after atropine alone ($P > 0.10$) in these two larval stages. In adults, no drug combination had any significant effect on resting f_H.

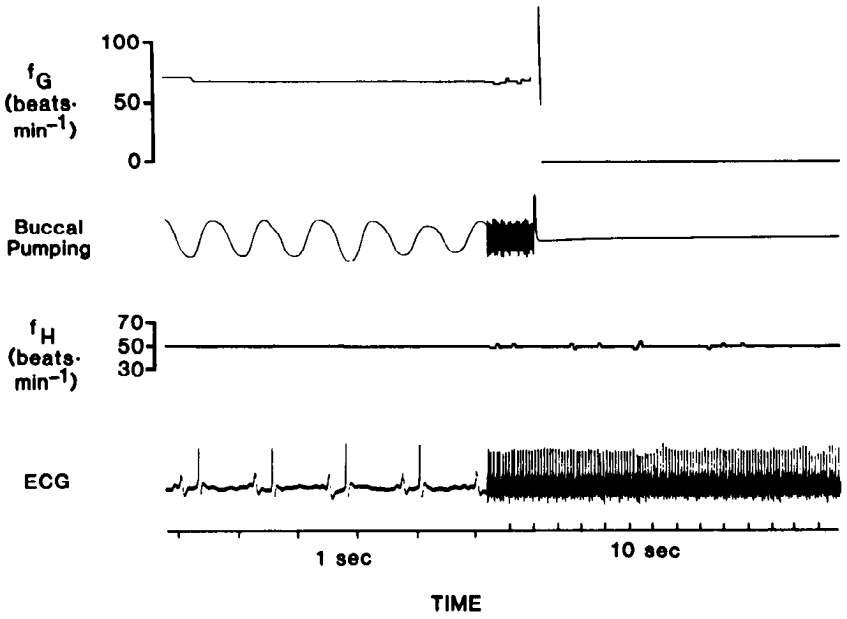

FIG. 3. Records of buccal ventilation and heart beat in 1.55-g *stage VI* larva of *Rana catesbeiana*, unrestrained and resting in normoxic conditions. Approximately halfway along record there was voluntary cessation of buccal ventilation. f_G, buccal ventilation; f_H, heart rate; ECG, electrocardiogram.

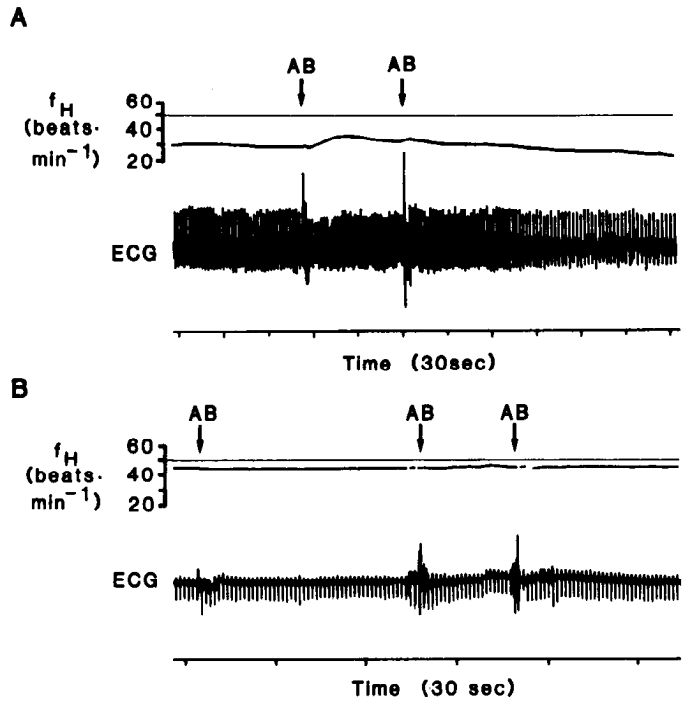

FIG. 4. Representative records of electrocardiogram (ECG) and heart rate (f_H) during voluntary air breaths (AB) in normoxia in unrestrained *Rana catesbeiana*. *A*, 82.80-g postmetamorphic adult; *B*, 3.21-g larva (*stage XX*). Each time mark represents 30 s.

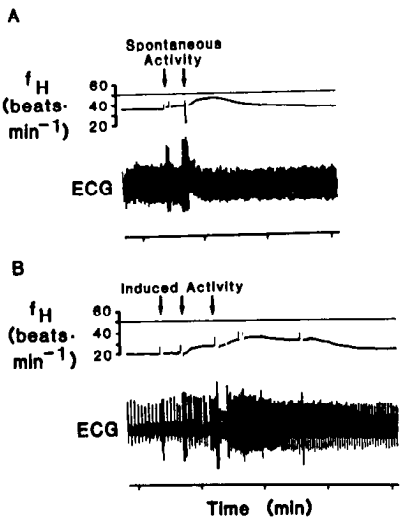

FIG. 5. Representative records of electrocardiogram (ECG) and heart rate (f_H) during activity in *Rana catesbeiana*. *A*, burst of voluntary spontaneous swimming in 2.44-g *stage XIV* larva; *B*, records during activity induced by mechanical stimulation in 7.09-g adult. Each time mark represents 1 min.

Heart Rate Responses to Activity

Influence of developmental stage. Spontaneous voluntary locomotor movements in otherwise resting animals were observed in all developmental stages. There was no change in f_H during such activity in newly hatched larvae. In all other developmental stages, however, movement of more than a few tail beats or limb movements usually caused an increase in f_H (Fig. 5*A*).

Intense activity induced experimentally by mechanical stimulation produced increases in f_H that were quite similar to those occurring during spontaneous movement, although not surprisingly the increases lasted longer than ones resulting from less extreme spontaneous activity (Fig. 5*B*). The effect of intense activity on f_H varied considerably with developmental stage (Fig. 6). Immediately after hatching, mean f_H after induced activity was not significantly different ($P > 0.10$) from that during rest. In all older developmental stages, including adults, mean f_H measured immediately after activity was significantly higher ($P < 0.01$) than either control or atropinized f_H at rest. The magnitude of the increase in f_H varied somewhat with developmental stage, being largest in adults, which showed a 75% increase (Figs. 6 and 7).

Effects of atropine and propranolol. The effects of cholinergic and β-adrenergic changes in f_H with activity were assessed in all but the youngest developmental group.

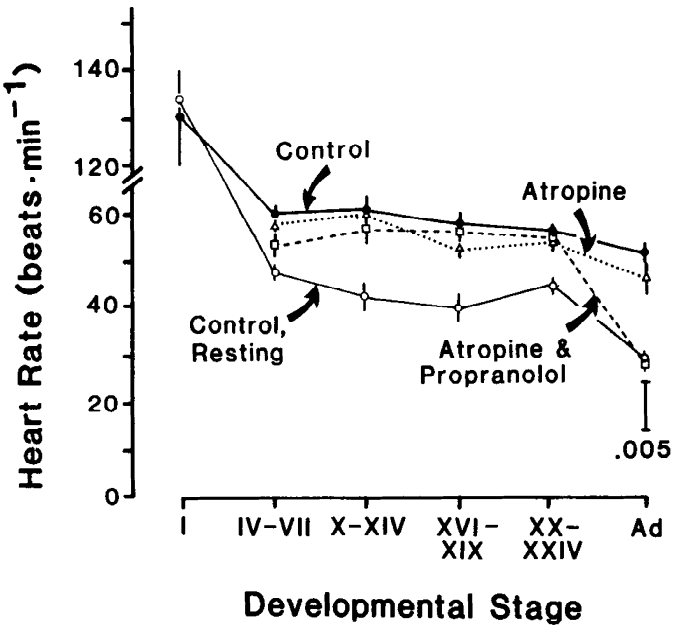

FIG. 6. Heart rate in unrestrained *Rana catesbeiana* in normoxia during intense activity, as a function of developmental stage before and after cholinergic (atropine) and combined cholinergic and β-adrenergic (atropine + propranolol) blockade. As a reference, values for resting control animals that were presented in Fig. 1 are replotted. Values are means ± SE. Number of animals for each development stage are as in Fig. 1. If ANOVA revealed significant differences between control, atropine, and atropine + propranolol treatments within single developmental group, then level of significance is indicated below mean values for that group. Also shown for that group is minimum significant difference for multiple unplanned comparisons, as described in legend of Fig. 1. See text for further details.

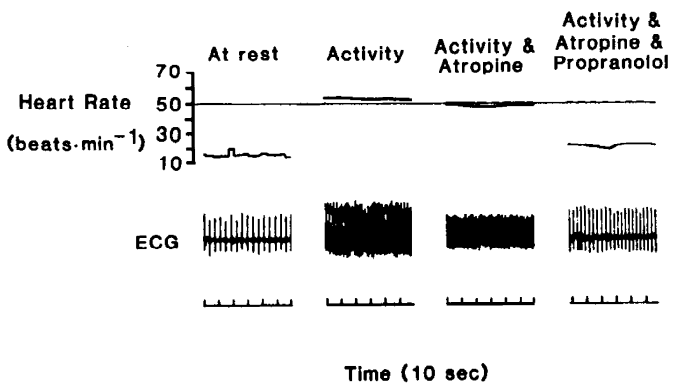

FIG. 7. Representative records of electrocardiogram (ECG) and heart rate in unrestrained 65.93-g adult *Rana catesbeiana*. Records were made when bullfrog was resting and immediately after intense activity under control conditions and after cholinergic or combined cholinergic and adrenergic blockade. Each time mark represents 10 s.

Surprisingly, in all larval stages examined, f_H after intense activity still increased to the same extent after ATR or ATR + PRO were administered (Fig. 6); i.e., a tachycardia after activity persisted even during cholinergic and β-adrenergic blockade. In adults, ATR alone had no significant effect on mean f_H after intense activity (Figs. 6 and 7). However, ATR + PRO completely eliminated the tachycardia after intense activity.

Effects of changing venous return. Experiments were designed to test whether the tachycardia after intense activity in *stage X–XIV* larvae and adults could result directly from changes in venous return associated with the body movements during activity. f_H typically increased 5–10 beats·min^{-1} within a few heart beats of the beginning of saline infusion, decreasing back toward the control rate after cessation of infusion (Fig. 8). The increases in f_H produced by infusion after cholinergic and combined cholinergic and β-adrenergic blockade were not statistically different from control increases (Table 1).

Heart Rate during Hypoxia and Hyperoxia

Under resting conditions, newly hatched larvae exposed to environmental hypoxia showed no significant change ($P > 0.10$) in f_H until a critical P_{O_2} of ~40–50 Torr, below which level f_H fell sharply by >60%. f_H during hyperoxia was not significantly different from normoxic values. Larval stages from *stage IV* and beyond as well as adults showed no significant change in resting f_H with either hyperoxia or hypoxia down to a P_{O_2} of 30 Torr, the lowest level tested.

Mean resting f_H after ATR and ATR + PRO was determined during hypoxic (30 Torr) and hyperoxic

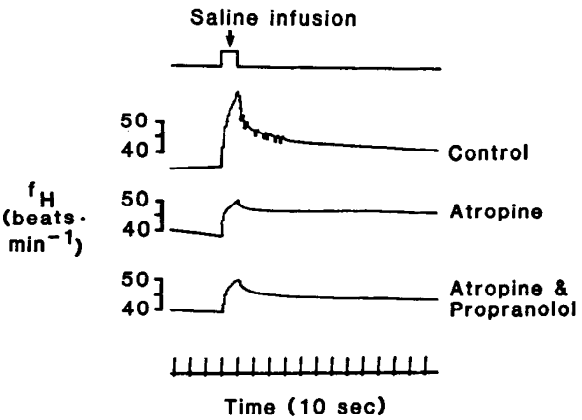

FIG. 8. Chronotropic responses of in situ heart of 6.13-g newly metamorphosed adult to saline infusion into central veins. Three sequential traces represent separate infusions under control conditions, after cholinergic blockade with atropine, and after combined cholinergic and β-adrenergic blockade with atropine and propranolol. Each time mark represents 10 s. This record shows typical rapid increase in heart rate (f_H) accompanying saline infusion but is somewhat atypical with respect to larger magnitude of response and its persistence after infusion was stopped. See text for details.

TABLE 1. *Direct effects on heart rate at 20°C produced by infusion of saline into central venous circulation of Rana catesbeiana*

	n	Mass, g	Increase in f_H, beats·min^{-1}			P
			Control	ATR	ATR + PRO	
Larvae (*stage V–VIII*)	5	2.0±0.2	9±3	6±3	6±1	>0.1
Adults	3	7.4±1.0	8±6	6±3	5±2	>0.1

Values are means ± SE. Central nervous system has been destroyed (see text for details). Last column shows P from one-way ANOVA of mean values for 3 separate conditions. f_H, heart rate; ATR, atropine; PRO, propranolol.

(>500 Torr) exposure. Essentially, all of the chronotropic effects induced by these drug combinations were not statistically different from the effects of these drugs on resting f_H under normoxic conditions. Thus, with the exception of severe hypoxia on the earliest larval stages, the level of inspired O_2 does not affect the normal responses of the heart to cholinergic or β-adrenergic blockade.

DISCUSSION

Regulation of Heart Rate at Rest

Resting f_H of the full-grown adult bullfrog is less than one-quarter that of the newly hatched larva, but the decline in f_H with development is clearly not a steady progression (Fig. 1). Detailed discussion of the mechanisms behind developmental changes in the absolute levels of resting f_H are largely beyond the scope of the present paper, for it involves not only consideration of development but also requires allometric analysis (W. Burggren, unpublished work). However, it is noteworthy that the fall in resting f_H during development in *R. catesbeiana* appears to result from changes in the intrinsic frequency of the cardiac pacemaker, rather than from developmental adjustments in tonic parasympathetic inhibition or sympathetic stimulation of f_H. This is inferred from the observation that, after combined cholinergic and β-adrenergic blockade (presumably eliminating tonic neural-hormonal influences on f_H), f_H remains much lower in adults than in any identically treated larval stage (Fig. 1).

A significant vagal inhibition of the heart was evident in resting larvae of *stages XVI–XIX*, since treatment with ATR produced a 20–25% increase in f_H. However, this vagal tone disappeared after metamorphosis. The influence of vagal parasympathetic fibers on f_H of amphibians is dependent on season (15), and this phenomenon could have accounted for the lack of vagal tone that we observed in resting undisturbed bullfrogs. Yet, the experiments on adults were conducted during the winter, spring, and summer, with findings consistent between seasons.

β-Adrenergic blockade in the absence of vagal inhibition (i.e, after treatment with atropine) had no effect on resting f_H at any stage, indicating little or no sympathetic stimulation above intrinsic rate. This interpretation conflicts with the earlier findings of Lillo (14) for adult *R. catesbeiana* with chronically implanted femoral arterial and abdominal venous catheters, in which β-adrenergic blockade reduced f_H by ~25%. However, the control f_H's for adult *R. catesbeiana* in Lillo's (14) study were significantly higher than we have observed in the present study, at the end of each of the several periods of normoxic resting recovery interspersed throughout the experimental protocol. Possibly, the more invasive experimental techniques of Lillo's (14) study might have resulted in chronic sympathetic stimulation, e.g., increase of circulating catecholamines, of f_H above that of the animals of the present study.

Adjustments of f_H associated with ventilatory events were conspicuously absent in any of the larval stages of *R. catesbeiana* (Fig. 4*B*). Even the temporary cessation of gill ventilation in larvae had no influence on f_H (Fig. 3). The constancy of f_H during air-breathing episodes has been previously noted for anuran larvae (6, 24). Clearly, the present experiments have revealed that potential effector mechanisms for adjusting f_H, e.g., decrease in vagal tone and sympathetic stimulation, do exist. Why, then, does f_H not change during adjustments in lung or gill ventilation in bullfrog larvae, given the importance of matching perfusion and ventilation in gas exchange organs? Three explanations are viable. It is possible that the central nervous system of the developing larva is insufficiently developed to modulate cardiovascular function during ventilatory adjustments. Alternatively, those peripheral reflexes involved in adjustment of f_H in adult amphibians (26, 27) may be absent or immature in larvae. Finally, it should be noted that the skin achieves the major share of both O_2 uptake and CO_2 elimination in larval *R. catesbeiana* until metamorphosis (6, 7). The absence of changes in f_H with adjustment in gill or lung ventilation in larvae may simply reflect the fact that during these experiments the conditions for gas exchange at the major gas exchange site (the skin) remained much more constant than in the intermittently ventilated lungs, for example. Thus overall gas exchange in larvae is probably best served by a relatively constant f_H and skin perfusion, irrespective of changes in gill or especially lung ventilation.

Ventilation tachycardia, i.e., an increase in f_H during voluntary episodes of lung or gill ventilation, has been described for many adult amphibians and reptiles that only intermittently breathe air or water (3, 11, 20) and has been observed for adult *R. catesbeiana* in the present study (Fig. 4*A*). However, the efferent mechanisms involved in changes in f_H during intermittent lung ventilation by which ventilation tachycardia in anurans is affected are not clear. Lillo (14) reported that the bradycardia during forced submergence of adult bullfrogs was mediated entirely by parasympathetic cardiac inhibition. However, Lund and Dingle (15) have shown that, although diving bradycardia produced by forced submersion of adult *R. pipiens* was tempered by atropinization or bilateral vagotomy, it was certainly not eliminated. In the present study, adjustments in f_H associated with intermittent voluntary lung ventilation in adult bullfrogs persisted after cholinergic blockade but were completely abolished by β-adrenergic blockade. It should be emphasized that the mechanisms behind cardiac responses during the onset of lung ventilation in voluntarily apneic adults may be quite different from those operating during recovery after forced submersion. Clearly, further experiments are required to elucidate the effector mechanism(s) for f_H adjustments during intermittent lung ventilation in adult anurans.

Regulation of Heart Rate During Activity

Tachycardia associated with activity in lower vertebrates generally results from the combined effects of

withdrawal of vagal inhibition, sympathetic neural stimulation, and excitation by circulating catecholamines (4, 13). The present study indicates for adult bullfrogs that the tachycardia after intense activity is mediated sympathetically rather than cholinergically, because it is unaltered by cholinergic blockade but completely eliminated by β-adrenergic blockade (Figs. 6 and 7). It is surprising, however, that the tachycardia after intense activity in larvae persists in the face of combined cholinergic and β-adrenergic blockade, because it is generally regarded that adjustments in rate of the vertebrate heart are mediated by cholinergic and β-adrenergic receptors (23). This observation could be explained in several ways. The tachycardia induced by activity in larvae might be mediated by a population of α-adrenergic receptors in the heart that remain functional during β-adrenergic blockade (1, 9, 10). A second possibility is that increased systemic venous return during activity in larvae may directly increase f_H by stretch of the pacemaker cells. The present experiments on both larval and adult bullfrogs reveal that direct mechanical stretch of the heart produced by central venous infusion can indeed increase f_H (Fig. 8). An increase in f_H due to the direct mechanical effects of stretch on the pacemaker cells has been shown for a wide variety of animals (including amphibians), and in mammals it persists after β-adrenergic blockade (10).

Regulation of Heart Rate During Hypoxia and Hypercapnia

With the single exception of extreme hypoxia in *stage I* larvae, acute changes in O_2 availability per se had no significant effect on f_H in any of the developmental stages examined, including adults. Previous experiments on adult *R. pipiens* (A. Pinder, unpublished observations), larval and adult *Xenopus laevis* (8, 18), and larval *R. catesbeiana* (25) similarly have shown f_H to be independent of O_2 availability. Adult *Bufo marinus*, however, show a tachycardia on hypoxic exposure (26). Clearly, there is no single universally appropriate f_H response to hypoxia in amphibians, particularly because changes in stroke volume could compensate for changes in f_H. The 50–60% fall in f_H as ambient P_{O_2} fell from 60 to 30 Torr in newly hatched larvae of *R. catesbeiana* could represent a reflex adjustment of f_H. However, because no other experimental perturbation of newly hatched larvae induced any change from control f_H in newly hatched larvae, it is possible that bradycardia during severe hypoxia represents a direct depression of cardiac function at this early developmental stage.

Summary

These experiments have revealed both qualitative and quantitative differences between larval and adult bullfrogs with respect to their ability to regulate f_H. Some of these differences simply reflect apparent larval immaturity of elements involved in cardiac regulation. It is important to emphasize, however, that cardiorespiratory processes in the later larval stages are more complex than in adults because of the two respiratory media and three sites for gas exchange organs that are used simultaneously by these larvae. Consequently, developing amphibians should not be viewed as having a linear rate of maturation and of elaboration of physiological regulatory mechanisms.

The authors appreciate the useful comments of Eric Bittman, Martin Feder, Peter Kimmel, Allan Smits, and Nigel West during the preparation of the manuscript.

This project was supported by National Science Foundation Grant PCM-8502724 and a University of Massachusetts Faculty Research Grant.

Present address of M. Doyle: Dept. of Physiology, School of Medicine, University of New Mexico, Albuquerque, NM 87131.

Received 11 July 1985; accepted in final form 24 February 1986.

REFERENCES

1. ASK, J. A., AND G. STENE-LARSEN. Functional α_1-adrenoceptors in the rat heart during β-receptor blockade. *Acta Physiol. Scand.* 120: 7–13, 1984.
2. BAGSHAW, R. J. Evolution of cardiovascular baroreceptor control. *Biol. Rev.* 60: 121–162, 1985.
3. BURGGREN, W. W. A quantitative analysis of ventilation tachycardia and its control in two chelonians *Pseudemys scripta* and *Testudo graeca*. *J. Exp. Biol.* 63: 367–380, 1975.
4. BURGGREN, W. W. *An Investigation of Gas Exchange and Cardiovascular Dynamics in the Chelonian Reptiles* (PhD thesis). East Anglia, UK: University of East Anglia, 1976.
5. BURGGREN, W. W. Transitions of respiratory processes during amphibian metamorphosis: from egg to adult. In: *Respiration and Metabolism in Embryonic Vertebrates*, edited by R. S. Seymour. The Hague: Junk, 1984.
6. BURGGREN, W. W., M. E. FEDER, AND A. W. PINDER. Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* 65: 263–273, 1983.
7. 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.
8. FEDER, M. E., AND R. WASSERSUG. Aerial versus aquatic oxygen consumption in larvae of the clawed frog, *Xenopus laevis*. *J. Exp. Biol.* 108: 231–245, 1984.
9. GORBMAN, A., W. W. DICKHOFF, S. R. VIGNA, N. B. CLARK, AND C. L. RALPH. *Comparative Endocrinology*. New York: Wiley, 1983.
10. IRISAWA, H. Comparative physiology of the cardiac pacemaker mechanism. *Physiol. Rev.* 58: 461–498, 1978.
11. JOHANSEN, K., AND W. W. BURGGREN. Cardiovascular function in the lower vertebrates. In: *Hearts and Heart-like Organs*, edited by G. H. Bourne. New York: Academic, 1980.
12. JOHANSEN, K., AND W. W. BURGGREN (Editors). *Cardiovascular Shunts: Phylogenetic, Ontogenetic and Clinical Aspects*. Copenhagen: Munksgaard, 1985.
13. JONES, D. R., AND D. J. RANDALL. The respiratory and circulatory systems during exercise. In: *Fish Physiology*, edited by W. S. Hoar and D. J. Randall. New York: Academic, 1978, vol. 7.
14. LILLO, R. S. Autonomic cardiovascular control during submergence and emergence in bullfrogs. *Am. J. Physiol.* 237 (*Regulatory Integrative Comp. Physiol.* 6): R210–R216, 1979.
15. LUND, G. F., AND H. DINGLE. Seasonal temperature influence on vagal control of diving bradycardia in the frog (*Rana pipiens*). *J. Exp. Biol.* 48: 265–277.
16. MOTT, J. C. Control of the foetal circulation. *J. Exp. Biol.* 100: 129–146, 1982.
17. QUINN, D., AND BURGGREN, W. Lactate production, tissue distribution and elimination following exhaustive exercise in larval and adult bullfrogs *Rana catesbeiana*. *Physiol. Zool.* 56: 309–318.
18. SHELTON, G. The effects of lung ventilation on blood flow to the lungs and body of the amphibian, *Xenopus laevis*. *Respir. Physiol.* 9: 183–196, 1970.

19. SHELTON, G. Gas exchange, pulmonary blood supply, and the partially divided amphibian heart. *Perspect. Exp. Biol.* 1: 247–259, 1976.
20. SHELTON, G., AND R. G. BOUTILIER. Apnoea in amphibians and reptiles. *J. Exp. Biol.* 100: 245–273, 1982.
21. SOKAL, R. F., AND F. J. ROHLF. *Biometery.* San Francisco, CA: Freeman, 1981.
22. TAYLOR, A. C., AND J. J. KOLLROS. Stages in the normal development of *Rana pipiens* larvae. *Anat. Rec.* 94: 7–24, 1946.
23. TAXI, J. Morphology of the autonomic nervous systems. In: *Frog Neurobiology*, edited by R. Llinas and W. Precht. New York: Springer-Verlag, 1981.
24. WEST, N. H., AND W. W. BURGGREN. Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpoles (*Rana catesbeiana*). *Respir. Physiol.* 47: 165–176, 1982.
25. WEST, N. H., AND W. W. BURGGREN. Reflex interactions between aerial and aquatic gas exchange organs in larval bullfrogs. *Am. J. Physiol.* 244 (*Regulatory Integrative Comp. Physiol.* 13): R770–777, 1983.
26. WEST, N. H., AND W. W. BURGGREN. Factors influencing pulmonary and cutaneous arterial blood flow in the toad, *Bufo marinus*. *Am. J. Physiol.* 247 (*Regulatory Integrative Comp. Physiol.* 16): R884–R894, 1984.
27. WEST, N. H., AND B. N. VAN VLIET. Open-loop analysis of the pulmocutaneous baroreflex in the toad *Bufo marinus*. *Am. J. Physiol.* 245 (*Regulatory Integrative Comp. Physiol.* 14): R642–R650, 1983.