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RESEARCH ARTICLE

Corticosterone promotes emergence of fictive air breathing in *Xenopus laevis* Daudin tadpole brainstems

Stéphanie Fournier, Pierre-Luc Dubé and Richard Kinkead*

Department of Paediatrics, Université Laval, Centre de Recherche du Centre Hospitalier Universitaire de Québec, Hôpital St-François d'Assise, 10 rue de l'Espinay, Québec City, QC, Canada, G1L 3L5

*Author for correspondence (richard.kinkead@crsfa.ulaval.ca)

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SUMMARY

The emergence of air breathing during amphibian metamorphosis requires significant changes to the brainstem circuits that generate and regulate breathing. However, the mechanisms controlling this developmental process are unknown. Because corticosterone plays an important role in the neuroendocrine regulation of amphibian metamorphosis, we tested the hypothesis that corticosterone augments fictive air breathing frequency in *Xenopus laevis*. To do so, we compared the fictive air breathing frequency produced by *in vitro* brainstem preparations from pre-metamorphic tadpoles and adult frogs before and after 1 h application of corticosterone (100 nmol l^{-1}). Fictive breathing measurements related to gill and lung ventilation were recorded extracellularly from cranial nerve rootlets V and X. Corticosterone application had no immediate effect on respiratory-related motor output produced by brainstems from either developmental stage. One hour after corticosterone wash out, fictive lung ventilation frequency was increased whereas gill burst frequency was decreased. This effect was stage specific as it was significant only in preparations from tadpoles. GABA application (0.001–0.5 mmol l^{-1}) augmented fictive air breathing in tadpole preparations. However, this effect of GABA was no longer observed following corticosterone treatment. An increase in circulating corticosterone is one of the endocrine processes that orchestrate central nervous system remodelling during metamorphosis. The age-specific effects of corticosterone application indicate that this hormone can act as an important regulator of respiratory control development in *Xenopus* tadpoles. Concurrent changes in GABAergic neurotransmission probably contribute to this maturation process, leading to the emergence of air breathing in this species.

Key words: GABA, hormone, amphibian, rhythm generation.

INTRODUCTION

The transition from water to air breathing that takes place during amphibian metamorphosis implies numerous and well orchestrated physiological transformations. During tadpole development, the gills regress while the lungs emerge as an important gas exchange structure; simultaneous vascular reorganisation improves gas exchange between the lungs and tissues. To be fully functional, however, these anatomical transformations must coincide with significant changes in motor control. Air breaths are occasional during early tadpole stages but, as development progresses, their frequency increases significantly, commensurate with the declining contribution of the gills to gas exchange (Burggren and Doyle, 1986).

The neural circuits responsible for the generation of gill and air breathing in bullfrogs are anatomically and functionally distinct entities that also undergo significant developmental changes (Broch et al., 2002; Galante et al., 1996; Vasilakos et al., 2006; Wilson et al., 2002; Winmill and Hedrick, 2003). In pre-metamorphic tadpoles, the neurons generating lung ventilation are located in a region immediately caudal to the Xth cranial nerve. As the animal matures, this rhythmogenic entity 'relocates' to a more rostral region located between the Vth and Xth cranial nerves (Torgerson et al., 2001). In post-metamorphic brainstems, the rhythmogenic circuit driving lung ventilation is located between cranial nerves VIII and IX (McLean et al., 1995; Wilson et al., 2002) whereas the neurons driving buccal activity (formerly gill ventilation) are located more caudally, at the level of the Xth cranial nerve (Wilson et al., 2002). In pre-metamorphic tadpoles, the specific location of the network generating gill breathing has not been investigated.

By demonstrating that superfusing tadpole brainstems with Cl^--free medium abolishes fictive gill ventilation but has minimal impact on lung-related motor output, Galante and colleagues provided the first direct evidence that gill and lung ventilation are driven by functionally distinct processes (Galante et al., 1996). Their results led them to conclude that the circuits generating gill ventilation require GABA/glycine neurotransmission. Although our understanding of the mechanisms driving lung ventilation remains relatively limited, the predominating hypothesis proposes that, in bullfrogs, lung ventilation matures from a voltage-dependent pacemaker-driven process (tadpoles) to a voltage-independent network-driven process (adults) (Winmill and Hedrick, 2003). This interpretation is consistent with data showing that the sensitivity of fictive lung ventilation to Cl^--free medium is greater in preparations from adult frogs than in those from tadpoles (Broch et al., 2002) and the concept that developmental changes in GABA/glycine neurotransmission (*via* changes in Cl^- regulation) contribute to the maturation of these neural networks (Ben-Ari et al., 2007; Ben-Ari et al., 1997).

In developing tadpoles, the brainstem neurons that generate air breathing are present well before the lungs become functional but their activity remains more or less suppressed until metamorphosis owing to GABA-dependent pathways (Straus et al., 2000). To this

day, the mechanisms regulating 'developmental disinhibition' of air breathing are not well understood but there is strong evidence indicating that the developmental changes in Cl^- gradients, which explain maturation of the post-synaptic effects of GABA (from excitatory to inhibitory), contribute to respiratory control maturation in amphibians. In bullfrog brainstems, application of low GABA or glycine concentrations augments lung burst frequency in tadpoles but has the opposite effect in adult frogs (Broch et al., 2002). Moreover, the developmental decrease in the expression of the $Na^+/K^+/Cl^-$ co-transporter NKCC1 plays a role in the maturation of the central O_2 chemoreflex recorded *in vitro* (Fournier and Kinkead, 2008). Yet, the mechanisms initiating and/or regulating these aspects of respiratory control development are unknown.

The shift from tadpole to adult life is initiated by environmental cues that trigger diverse neuroendocrine responses including stimulation of the inter-renal glands. Following their release into the bloodstream, corticosteroids act in synergy with thyroid hormone to promote extensive remodelling of the central nervous system (Denver, 1998). Given this role and the fact that, in rats, the neuroendocrine response to stress affects Cl^- homeostasis and GABAergic neurotransmission (Hewitt et al., 2009), it is plausible that corticosterone affects the respiratory control system to augment air breathing frequency in developing tadpoles. To test this hypothesis, we compared the fictive air breathing frequency produced by *in vitro* brainstem preparations from adult and pre-metamorphic *Xenopus* before and after 1 h application of corticosterone; the effect of corticosterone on the responsiveness to GABA application was also compared in both age groups.

MATERIALS AND METHODS

Animals

Experiments were performed on 31 brainstem preparations from *Xenopus laevis* Daudin tadpoles and 14 adult frogs. Adults were obtained from a commercial supplier (Nasco, Fort Atkinson, WI, USA); some were used in experiments whereas others were bred to produce tadpoles. Animals were housed in aquaria supplied with flowing, filtered and dechlorinated Québec City water maintained between 21 and 24°C (12 h:12 h light/dark photoperiod). *Xenopus laevis* were fed with dry food from Nasco. 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. Quantification of the neural correlates of gill and lung breathing in this species was described recently (Kimura, 2010); this approach and the motor output produced by this brainstem preparation are nearly identical to that observed in bullfrogs (Kinkead et al., 1994; McLean and Remmers, 1997).

In vitro brainstem preparations

Animals were anaesthetised by immersion in a solution of tricaine methane sulphonate (1 g l^{-1}) buffered to pH 7.0 with $NaHCO_3$. The beaker was then placed on ice for 20–30 min to slow metabolism and ensure adequate anaesthesia throughout the dissection (Winmill and Hedrick, 2003). Once unresponsive to body pinch, tadpoles and frogs were decerebrated by a transection just rostral to the eyes. Tadpoles were then placed under the dissection microscope for determination of the developmental stage based on the criteria of Nieuwkoop and Faber (Nieuwkoop and Faber, 1994); animals between stages 51 and 56 were used. The cranium was opened to expose the brainstem and rostral spinal cord, and to allow dissection of the cranial nerves. The brain was irrigated with ice-cold (0–5°C) artificial cerebrospinal fluid (aCSF) to avoid a sudden change in temperature and reduce axonal conductance throughout the dissection procedure (Fournier et al., 2007). The composition of the aCSF was identical to that developed for *Xenopus laevis* (Zornik and Kelley, 2008) and consisted of (mmol l^{-1}): NaCl 75.0, KCl 2.0, $MgCl_2$ 0.5, D-glucose 11.0, $NaHCO_3$ 25.0 and $CaCl_2$ 2.0. The composition of the aCSF used for brainstem superfusion was the same for tadpoles and adults. The superfusate was equilibrated with a 98% O_2/2% CO_2 gas mixture and had a pH of 7.90±0.15. The brainstem 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, Midland, MI, USA), where it was immobilised with insect pins. The arachnoid and pia membranes were carefully removed and the brain was moved to the recording chamber where it was placed ventral side up.

Electrophysiological recordings

Cranial nerve burst amplitude from a single electro-neurogram is not always sufficient to adequately identify fictive lung and buccal bursts (Sanders and Milsom, 2001). Thus, using suction electrodes, bursts of respiratory-related motor activity were recorded simultaneously from the rootlets of the trigeminal (V) and vagal (X) nerves. Vagal nerve activity was used as a sensitive marker of fictive lung activity to distinguish between lung- and buccal-related signals (Kogo et al., 1994; Kogo and Remmers, 1994).

The pipettes were constructed from borosilicate glass (0.84 mm i.d.) pulled to a fine tip with a vertical microelectrode puller (Stoelting Co., Wood Dale, IL, USA). The tip was broken and bevelled to achieve an appropriate tip diameter. Neural activity signals recorded from the suction electrodes were amplified (gain 10,000) and filtered (low cut-off 10 Hz, high cut-off 1 kHz) using a differential AC amplifier (model 1700; A-M Systems, Everett, WA, USA). Vagal and trigeminal signals were then full-wave rectified and integrated (time constant 100 ms) using a moving averager (model MA-821; CWE, Ardmore, PA, USA). The raw and integrated nerve signals were viewed on an oscilloscope and digitised for recording with a data acquisition system (model DI-720; Dataq Instruments, Akron, OH, USA). The sampling rate of the analog to digital conversion for the raw signal was 2500 Hz.

Experimental protocols

Once the recording electrodes were in place, the brainstem preparation was superfused with aCSF at room temperature (20–22°C) delivered at a rate ranging between 7 and 10 ml min^{-1}. The aCSF was not recycled. The preparation was allowed to return to ambient temperature and stabilise until stable rhythmic neural activity was recorded from both nerves. This stabilisation period lasted ~60 min. Fig. 1 illustrates the time course of the experimental protocols used in the two series of experiments.

Series I: effects of corticosterone application on fictive gill and lung breathing frequency

Once recovery from dissection was complete and motor output appeared stable, baseline gill and lung breathing were recorded for 10 min (baseline 1; pre-corticosterone). Corticosterone (100 nmol l^{-1}) was applied to brainstem preparations from tadpoles (N=13) and adult frogs (N=7) for 60 min before a second (post-corticosterone) baseline recording was performed. The aCSF containing corticosterone was delivered continuously (non-static) at the same flow rate as the control aCSF. Note that corticosterone was first dissolved in DMSO (0.5 mmol l^{-1}); addition of the stock corticosterone solution into the main reservoir did not affect the pH of the aCSF. This corticosterone concentration was chosen because it corresponds to plasma levels measured in intact *Xenopus* (adults) following stress exposure (Yao

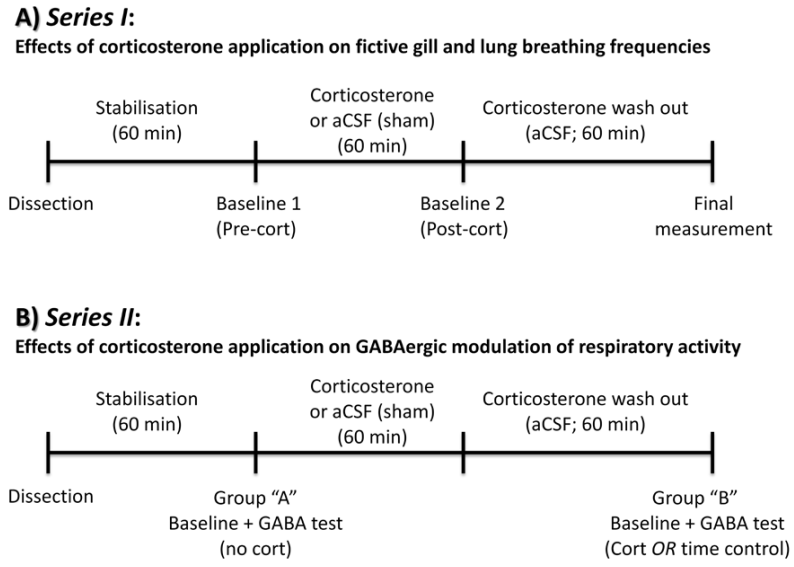

Fig. 1. Time course of the experimental protocols used in (A) series I on the effects of corticosterone application on fictive gill and lung breathing frequencies and (B) series II on the effects of corticosterone application on GABAergic modulation of respiratory activity. Note that in series II, the GABA test without corticosterone exposure was either performed immediately after the stabilisation period (group A; *N*=6) or after the entire protocol (group B, time control; *N*=5). Because data did not differ between groups, the results were pooled and compared with those obtained following corticosterone exposure. aCSF, artificial cerebrospinal fluid.

et al., 2004). The duration of corticosterone exposure was chosen arbitrarily based on results from preliminary experiments showing that this protocol had no acute effect but was sufficient to elicit long-term changes in respiratory motor output.

Considering the relatively long duration of the protocol (over 2 h), time-control experiments were performed to ensure that changes in fictive breathing were due to experimental treatment rather than to non-specific, time-dependent changes in motor output. In these experiments, the preparations from 13 tadpoles and 5 adult frogs were superfused with aCSF alone (i.e. without corticosterone application) for 2 h (Fig. 1; see below).

Series II: effects of corticosterone application on GABAergic modulation of respiratory activity

Tadpoles

These experiments first measured the changes in fictive gill and lung burst frequency that occurred in response to increasing GABA concentrations in the aCSF without previous exposure to corticosterone (*N*=11). Measurements were performed either immediately after the stabilisation period (group A; *N*=6) or after the entire (2 h) protocol (group B, time control; *N*=5). Following baseline measurement (0 mmol l^{-1}), five concentrations were used: 0.001, 0.005, 0.01, 0.1 and 0.5 mmol l^{-1}. The preparation was successively exposed to each concentration for 20 min without recovery between each dose. These doses were selected to facilitate comparison with previous reports (Broch et al., 2002; Galante et al., 1996). As the response to GABA measured in group A did not differ from that measured in group B (*P*=0.1), these data were pooled to increase statistical power and optimise the use of animals.

The effects of corticosterone exposure on the gill and lung burst responses to GABA application were then tested on seven brainstems that were first subjected to the corticosterone application protocol described for series I. In these experiments, the fictive breathing response to GABA was assessed at the end of the 1 h wash-out period.

Adult frogs

To determine whether the effects observed in tadpoles were age dependent, these experiments were also performed on adult frogs (*N*=7 control, *N*=7 post-corticosterone). Except for two preparations in which the response to GABA was measured immediately after the first stabilisation period (control), these experiments were performed at the end of the protocol described in series I (same animals). However, only the highest GABA concentration was tested (0.5 mmol l^{-1}).

Data analysis

Frequency values for respiratory burst activity were obtained by analysing the last 10 min of each condition (including baseline); the results were averaged for a 1 min period. Much like in bullfrogs, *in vitro* tadpole brainstem preparations from *Xenopus* typically produce two patterns of respiratory-related neural activity: (1) high frequency, low amplitude, and (2) low frequency, high amplitude, reflecting fictive buccal and lung ventilation, respectively (Liao et al., 1996; Torgerson et al., 1998). Unlike in bullfrogs, however, brainstem preparations from adult *Xenopus* rarely produce high frequency (buccal-related) activity. Although Kimura provides a good description of respiratory-related activity produced by *Xenopus* brainstems (Kimura, 2010), there is no thorough study of the relationship between the neural activity produced by the preparation and respiratory movements in intact or semi-intact animals. The rationale for determining that the motor output recorded was respiratory related is detailed in the critique of the methods given in the Discussion.

All measurements are reported as means ± 1 s.e.m. The results were analysed statistically using analysis of variance (ANOVA; Statview version 5.01, SAS Institute, Cary, NC, USA). Four factors influencing gill and lung burst frequencies were considered in the analysis: time (baseline 'pre-corticosterone' *versus* baseline 'post-corticosterone' *versus* wash out), corticosterone (with or without exposure), development (tadpoles *versus* adults) and GABA (0, 0.001, 0.005, 0.01, 0.1 and 0.5 mmol l^{-1}). Depending upon the analysis, the number of factors varied between 1 and 3, and a repeated measures design was used when appropriate. The ANOVA was followed by Fisher's protected least significant difference test ($P<0.05$) whenever results justified a *post hoc* test. *P*-values reported in the text are the results of ANOVA. Results from *post hoc* tests are displayed as symbols in the figures.

RESULTS

Age-dependent effects of corticosterone exposure on respiratory motor output

Based on the description provided by Kimura (Kimura, 2010), the main motor output produced by *Xenopus* brainstems consisted of

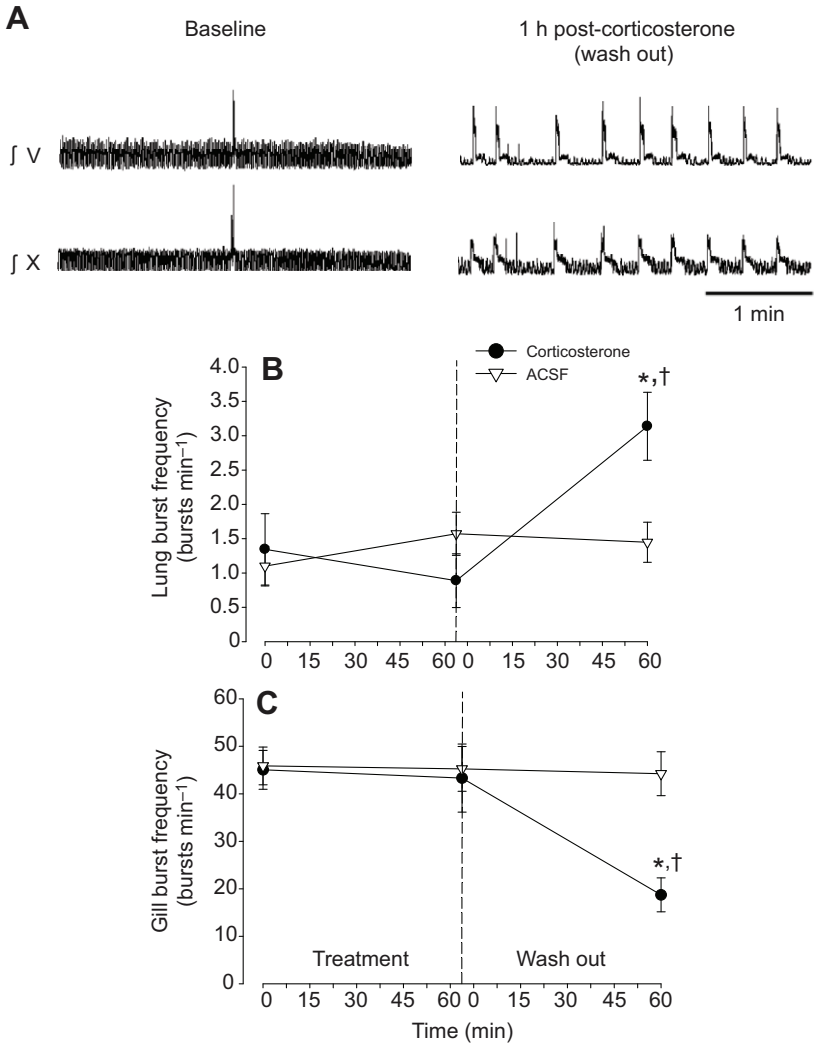

Fig. 2. (A) Integrated trigeminal (V) and vagal (X) neurograms comparing the motor output produced by a tadpole brainstem preparation before (baseline) and 1 h after corticosterone wash out. (B) Lung burst frequency before and after bath application of corticosterone (100 nmol l^{-1}) onto brainstem preparations for 1 h. The last data point shows the burst frequency measured 60 min after corticosterone wash out. These data are compared with the lung burst frequency measured in preparations superfused with aCSF (time control) throughout the protocol. (C) Fictive gill breathing frequency during and after corticosterone application. These data are compared with those obtained from time control preparations (aCSF). Data are reported as means ± s.e.m. *Significantly different from the first post-corticosterone measurement (baseline 2) at $P<0.05$. †Significantly different from the corresponding aCSF value (time control) at $P<0.05$.

respiratory-related activity similar to that described previously in preparations from bullfrogs (Torgerson et al., 1998). For tadpoles, respiratory motor output consisted mainly of fictive gill ventilation characterised by high frequency, low amplitude neural discharge (Fig. 2A). Fictive air breathing frequency was low and remained constant during corticosterone application but increased by more than two times during the 1 h wash-out period (Fig. 2B). This effect was specific to treatment (time × corticosterone: $P=0.002$) as maintaining the preparation in aCSF over a 2 h period without corticosterone exposure (time control) did not reveal time-dependent changes in lung burst frequency (Fig. 2B). Similarly, fictive gill ventilation remained unchanged during corticosterone application but decreased progressively during the wash-out period (Fig. 2C). Time control experiments showed that gill burst frequency was constant over a 2 h period; thus, the decrease was corticosterone related (time × corticosterone: $P=0.004$). The trigeminal and vagal neurograms presented in Fig. 2A illustrate these changes in burst pattern.

Despite suggestive trends, the lung burst frequency of adult brainstems was unchanged during corticosterone application or the wash-out period that followed (Fig. 3; time effect: $P=0.5$ and 0.19, respectively). ANOVA confirmed that the increase in lung burst frequency that takes place following corticosterone application is stage specific (corticosterone × development: $P=0.005$). As the neurograms presented in Fig. 3A show, fictive 'buccal' ventilation was rarely observed in preparations from adult frogs and could not be quantified appropriately.

Corticosterone exposure affects lung burst response to GABA

In tadpole brainstems that were not exposed to corticosterone, addition of GABA to the superfusion medium augmented fictive air breathing frequency (Fig. 4A). Corticosterone exposure reduced this response (Fig. 4A; GABA × corticosterone: $P=0.0006$). Similar results were obtained for fictive gill breathing. Specifically, GABA reduced gill burst frequency before but not after corticosterone exposure (Fig. 4B: GABA × corticosterone: $P=0.0007$).

In preparations from adult frogs, bath application of GABA (0.5 mmol l^{-1}) without prior corticosterone exposure reduced fictive air breathing frequency. However, this response was no longer observed following corticosterone application (Fig. 5). ANOVA on absolute frequency data confirmed that the effect of corticosterone on the fictive air breathing response to GABA (0.5 mmol l^{-1}) was age dependent (GABA × corticosterone × development: $P=0.0003$).

DISCUSSION

The results obtained in this study support our initial hypothesis as they showed that corticosterone application to reduced brainstem preparations augments the frequency of bursting events associated with air breathing in preparations from *Xenopus* tadpoles but not

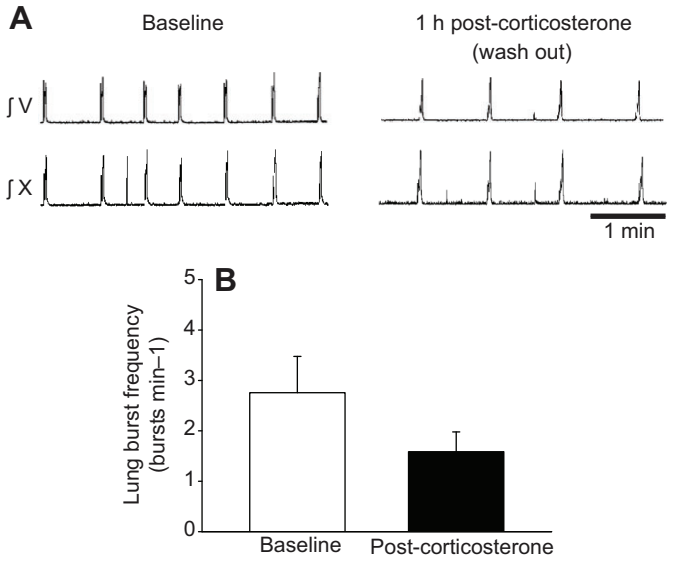

Fig. 3. (A) Integrated trigeminal (V) and vagal (X) neurograms comparing the motor output produced by an adult *Xenopus* brainstem before (baseline) and after bath application of corticosterone (100 nmol l^{-1} for 1 h). (B) Mean fictive air breathing frequency measured before and after corticosterone treatment. Data reported are means + s.e.m.

adults. As tadpole brainstems produced more fictive 'air breaths', the frequency of bursting events related to gill ventilation decreased. These results are important because they provide new insight into the mechanisms regulating the maturation of neurological processes leading to the emergence of air breathing in this group of vertebrates. In addition, the age-dependent nature of the effects suggests that beyond tadpole stages, the responsiveness of brainstem networks to 'acute' increases in corticosterone is limited. Finally, the demonstration that, in tadpole preparations, GABA no longer stimulates fictive air breathing following corticosterone exposure points to a key mechanism underlying maturation of the respiratory control system during amphibian development.

Critique of methods – is the motor output produced by the *Xenopus* preparation 'respiratory related'?

Unlike for bullfrogs (Gdovin et al., 1998; Gdovin et al., 1999), there are no rigorous studies of the neural correlates of breathing in reduced preparations from *Xenopus*. However, we are confident that the motor output recorded is respiratory related for several reasons. First, the neuroanatomical organisation of the neural network generating breathing in adult *Xenopus* is similar to that of bullfrogs (Zornik and Kelley, 2007). Second, because they have adopted an entirely aquatic lifestyle, vocal calls in *Xenopus* do not require air flow. These movements are thus produced independently of breathing and use a single set of muscles (Zornik and Kelley, 2008). Finally, this simplified organisation, and the fact that the duration of compound action potentials associated with calling is typically 20 ms (Yamaguchi and Kelley, 2000), whereas the average air breath lasts roughly 2 s, makes it easy to distinguish the two types of event. Admittedly, these distinctions do eliminate the possibility that the motor output recorded is related to or influenced by other activities such as swallowing or vomiting. Should these behaviours occur *in vitro*, we assume that the possibility of the related motor output being confused with breathing is limited.

Mechanisms of action of corticosterone

Recent evidence shows that corticosteroids can exert rapid, non-genomic effects on neurons *via* activation of G protein-coupled membrane receptors that can initiate downstream signalling cascades. Activation of these receptors exerts a vast array of rapid effects on different cells and tissues as well as on behavioural responses in different vertebrate species (Tasker et al., 2006). As no change in motor output was detected during corticosterone application, it would appear that the increase in fictive air breathing observed in tadpoles during the 'post-corticosterone' wash out was mediated *via* 'classical' steroid mechanisms involving transcriptional regulation. The ensuing effects are potentially numerous and in mature systems it has been shown that corticosteroids influence GABAergic tone by changing the subunit composition of $GABA_A$ receptors (Orchinik et al., 2001; Orchinik et al., 1994). Corticosterone application elicited significant changes in the fictive breathing response to GABA; however, as our experimental protocol did not assess the effects of GABA on fictive breathing in the presence of corticosterone, it is difficult to comment on the mechanisms by which corticosterone affected this system (genomic *versus* membrane receptors). Despite this limitation, the important changes in the fictive breathing response to GABA application bring us to propose that corticosterone induced modifications that are beyond the constitution of GABA receptors. Data showing that, in tadpoles, corticosterone application nearly reversed the effect of GABA on lung burst frequency strongly suggest that this hormone influences cellular processes responsible for maturation of GABAergic neurotransmission.

Chloride gradients are not established in immature neurons. During early life, the opening of Cl^- channels that takes place during GABA receptor activation therefore results in cell depolarisation. As the system matures, GABA becomes inhibitory owing to the

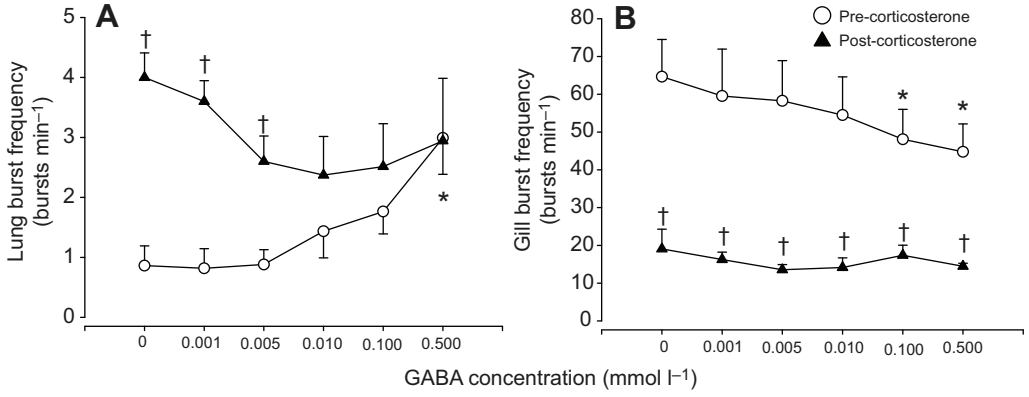

Fig. 4. Fictive (A) lung and (B) gill breathing frequency responses to increasing GABA concentration within the solution superfusing brainstem preparations from *Xenopus laevis* tadpoles. Each panel compares the responses measured without corticosterone exposure and 1 h after corticosterone treatment (100 nmol l^{-1} for 1 h). Data are expressed as means + s.e.m. *Significantly different from control condition (0 mmol l^{-1} GABA) at $P<0.05$. †Significantly different from the corresponding pre-corticosterone value at $P<0.05$.

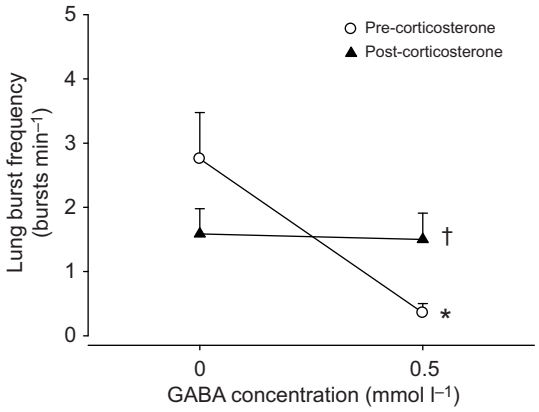

Fig. 5. Comparison of the fictive lung breathing response to bath application of GABA (0.5 mmol l^{-1}) measured without corticosterone application and 1 h after corticosterone treatment (100 nmol l^{-1} for 1 h). Experiments were performed on brainstem preparations from adult *X. laevis* frogs. Data are reported as means ± s.e.m. *Significantly different from control (0 mmol l^{-1} GABA) at $P<0.05$. †Significantly different from the corresponding pre-corticosterone value at $P<0.05$.

delayed expression of a chloride exporter that leads to a negative shift in the reversal potential for chloride ions (Ben-Ari, 2002). The K^+–Cl^--coupled co-transporter KCC2 extrudes Cl^- ions from the intracellular milieu. During development, its progressive expression plays an important role in the switch from GABA-mediated excitation to inhibition and contributes to maturation of the neural networks regulating breathing in bullfrog tadpoles (Fournier et al., 2007; Fournier and Kinkead, 2008). The processes regulating KCC2 expression during development are not entirely understood (Ben-Ari, 2002); however, the results obtained here bring us to propose that corticosterone may play an important role in this regard.

In bullfrogs, the loss of an inhibitory ($GABA_B$-mediated) signal during development is responsible for the ontogenic expression of air breathing. As Straus and colleagues mentioned in their discussion, this 'developmental disinhibition' may act *via* reductions in excitation, if the excitation (by exciting a functionally inhibitory pathway) leads to the suppression of behaviours (Straus et al., 2000). This interpretation is supported by the present work and results obtained in rats showing that the transition from excitatory to inhibitory is an important mechanism in the maturation of respiratory rhythmogenesis (Ren and Greer, 2006). Our study indicates that corticosterone may be an important signal in the initiation of this maturation process that seems highly conserved amongst vertebrates.

Conclusion and perspective

During metamorphosis, the central nervous system of tadpoles undergoes substantial remodelling. Some features that are exclusive to tadpole stages (e.g. lateral line system) are lost. By contrast, some functions gain in importance (e.g. limb motoneurons) whereas some cells performing one function as tadpoles are 'reworked' during metamorphosis to perform a new function in adults (e.g. motor trigeminal cells) (Denver, 1998; Lanoo, 1999). To ensure a successful transition from water to air breathing, such changes must occur within the neural networks that generate and regulate the act of breathing. While the anatomical and functional reorganisation of the respiratory control system of bullfrogs has been detailed elegantly (Galante et al., 1996; Straus et al., 2000; Vasilakos et al., 2006; Wilson et al., 2002), the mechanism responsible for initiating such changes is unknown. Environmental cues trigger the secretion of several hormones including thyroxin and corticosterone. Their synergistic action initiates a coordinated series of changes in virtually every tissue, which ultimately leads to metamorphosis (Denver, 1998). By demonstrating that corticosterone augments fictive air breathing frequency in tadpole brainstems, the present study strongly suggests that the development of the respiratory control system is also under hormonal control and that maturation of the GABAergic system is an important mechanism in that process. Clearly, several other mechanisms are likely to contribute to the changes in respiratory activity reported here. Given the strong similarities in the mechanisms that regulate diverse rhythmic motor behaviours, the developmental changes in locomotion that take place during *Xenopus* metamorphosis can provide valuables cues in this regard. For instance, exogenous nitric oxide (NO) application facilitates locomotor activity in adult frogs, an effect that contrasts with the inhibition of swimming at earlier larval stages (Sillar et al., 2008). But in the present context, it is interesting to note that NO modulates swimming by facilitating the release of GABA and glycine (Sillar et al., 2008). Nevertheless, the fact that NO also depolarises motoneurons and decreases membrane conductance (Sillar et al., 2008) points to an additional mechanism that deserves further investigation.

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