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THE EFFECTS OF BRANCHIAL DENERVATION AND PSEUDOBRANCH ABLATION ON CARDIOVENTILATORY CONTROL IN AN AIR-BREATHING FISH

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Summary

The role of sensory afferent information from the gills of *Amia calva* in cardiovascular and ventilatory control was investigated by bilateral branchial denervation and pseudobranch ablation.

Aquatic hypoxia or 1 mg of sodium cyanide (NaCN) in the water flowing over the gills stimulated bradycardia, and gill and air ventilation in sham-operated fish. Sodium cyanide, noradrenaline (NA) and adrenaline (A) infusion into the dorsal aorta increased gill ventilation, and NA and A infusion also stimulated tachycardia and an increase in blood pressure.

Following denervation and pseudobranch ablation, O_2 consumption ($\dot{V}_{O_2}$), air-breathing frequency (f_{AB}) and arterial O_2 tension (Pa_{O_2}) declined, and circulating NA levels increased, as compared with sham-operated fish. Cardiovascular and air-breathing responses to hypoxia were abolished and gill ventilatory responses attenuated. All ventilatory and cardiovascular responses to NaCN were abolished and gill ventilatory responses to NA and A were attenuated in animals following denervation and pseudobranch ablation.

These results demonstrate that O_2-sensitive chemoreceptors in the gills and pseudobranch control reflex bradycardia and air-breathing responses in *Amia*, but that gill ventilatory responses to hypoxia, NA and A are partially mediated by extrabranchial mechanisms. Plasma NA levels increased during hypoxia in sham-operated and denervated animals, indicating that circulating NA may have mediated gill ventilatory responses in denervated animals.

Introduction

Amia calva is a primitive fish with the ability to breathe both air and water. It

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possesses a swimbladder adapted to function as an air-breathing organ and gills for water-breathing. Relatively little is known about control of reflex ventilatory and cardiovascular responses to respiratory gases in *Amia* or air-breathing fish in general. Air-breathing and gill ventilation are stimulated by aquatic hypoxia in *Amia* (Johansen *et al.* 1970; Randall *et al.* 1981), and ventilatory responses to blood hypercapnia and acidosis do not occur unless they are associated with a reduction in blood O_2 content (McKenzie *et al.* 1991). This indicates that ventilatory control in *Amia* is similar to that observed in purely water-breathing fish, where blood and water O_2 status are the primary stimuli for cardioventilatory reflex responses (Randall and Jones, 1973; Smith and Jones, 1981; Randall, 1982).

There is much evidence indicating that hypoxic reflexes in fish are mediated by O_2-sensitive chemoreceptors in the gills and pseudobranch, innervated by cranial nerves VII, IX and X. Nerve activity sensitive to internal and external O_2 levels has been recorded from nerve X to the first gill arch of tuna (Milsom and Brill, 1986) and nerve IX to the first gill arch of trout (Burleson and Milsom, 1990).

Section of cranial nerves IX and/or X to the gill arches abolishes reflex bradycardia in response to aquatic hypoxia or NaCN (a potent chemoreceptor stimulant) in various teleost species (Smith and Jones, 1978; Fritsche and Nilsson, 1989; Burleson and Smatresk, 1990*a*). In elasmobranchs, nerves V and VII innervating the buccal cavity also require sectioning (Butler *et al.* 1977).

Complete branchial denervation abolishes ventilatory reflex responses to hypoxia in anaesthetised, spontaneously ventilating *Ictalurus punctatus* (Burleson and Smatresk, 1990*a*) and *Lepisosteus oculatus*, an air-breathing fish (Smatresk, 1991). The failure of branchial denervation to abolish ventilatory reflex responses completely in various other species (Hughes and Shelton, 1962; Saunders and Sutterlin, 1971) has been attributed to the existence of an extrabranchial O_2-sensitive chemoreceptor (Bamford, 1974) or to afferent information arising from the pseudobranch and/or nerves serving one particular gill arch, which were left intact (Smatresk, 1991). In channel catfish, O_2 sensitivity is located diffusely throughout the gills, and one branchial nerve left intact unilaterally is sufficient to stimulate a ventilatory response (Burleson and Smatresk, 1990*a*).

Recent evidence, however, suggests that ventilatory responses to hypoxia and acidosis in dogfish and trout may be mediated humorally by the circulating catecholamines NA and A, possibly *via* a direct effect on central respiratory motoneurones (Randall and Taylor, 1989; Taylor and Randall, 1988; Aota *et al.* 1990). There is evidence to suggest that circulating catecholamines play a role in gill ventilatory responses to acidosis in *Amia* (McKenzie *et al.* 1991).

The present study in *Amia* employed total branchial denervation (branchial branches of cranial nerves IX and X) and pseudobranch ablation to investigate the role of O_2-sensitive chemoreceptors in the gills and pseudobranch in reflex cardiovascular, air-breathing and gill ventilatory responses to hypoxia, sodium cyanide (NaCN) and catecholamines, and also to investigate the role of circulating catecholamines in reflex gill ventilatory responses to hypoxia. Sodium cyanide was used as a stimulant for O_2-sensitive chemoreceptors (Lahiri *et al.* 1970; Burleson

and Smatresk, 1990*a*; Smatresk, 1986). A conscious animal preparation was used to avoid the effects of anaesthesia, which may compromise cardiovascular and ventilatory reflex responses.

Materials and methods

Experimental animals

Amia weighing 400–1000 g were netted in Lake Erie and air-freighted to UBC, where they were maintained in a large outdoor fibreglass tank supplied with dechlorinated Vancouver tapwater (pH approx. 6.5, 7–12 °C). Fish were fed a diet of live goldfish and salmon fry. Before experimentation, *Amia* were placed in Plexiglas holding tanks and the water temperature was raised to 20 °C, over a 3- to 5-day period, to increase air-breathing frequency (Johansen *et al.* 1970).

Animal preparation

Animals were anaesthetized in a tricainemethanesulphonate (MS-222) solution at a concentration of 0.01 %, buffered with sodium bicarbonate. Following transfer to an operating table, fish were artificially ventilated with a buffered MS-222 solution at 0.005 %, oxygenated with 100 % O_2 to prevent hypoxia during surgery when only half of the gills were ventilated. A dorsal aortic cannula (PE 50, Intramedic) was implanted using the technique of Soivio *et al.* (1972). A buccal cannula was fitted using flared PE 50 (Intramedic) passed through a small hole in the roof of the mouth and secured with a cuff and sutures. An opercular cannula was fitted using flared PE 190 (Intramedic) passed through a small hole in the operculum and secured with a cuff and sutures. The operculum was reflected forward and a small incision made in the epithelium dorsal and posterior to the fourth gill arch. This allowed access to the branchial branches of cranial nerve X, serving all four gill arches. These were gently dissected free of connective tissue and sectioned with iris scissors, care being taken not to damage gill vasculature or musculature. Cardiac and visceral branches (including that to the swimbladder) of nerve X were left intact. The incision was closed with absorbable sutures (Ethicon 2.0). Cranial nerve IX serving the first gill arch was exposed by making a small incision at the base of the gill filaments where the arch meets the roof of the opercular cavity, and dissecting the nerve free of connective tissue. The nerve was then sectioned with iris scissors. The pseudobranch in *Amia* is glandular and situated in the roof of the mouth just anterior to the first gill arch. It was exposed *via* a small incision and removed by cautery. The same procedure was followed on the other side so that denervation and pseudobranch ablation were bilateral. Surgery took between 30 and 40 min and all nerve sections were confirmed *post mortem*, by dissection. Sham-operated animals had gill nerves exposed as described, but not sectioned.

Following surgery, fish were transferred to individual darkened Plexiglas chambers, ventilated with water until ventilatory movements resumed and then allowed to recover for 48 h with a continuous flow of aerated water (approx.

900 ml kg^{-1} min^{-1}) through the chamber. The anterior end of the chamber had an air space to allow air-breathing. The dorsal aortic cannula was flushed twice daily with heparinised saline.

Protocol

Following recovery, oxygen consumption ($\dot{V}_{O_2}$) was measured in both denervated and sham-operated fish (see below for analytical methods). Subsequently, the water-filled opercular cannula was attached to a pressure transducer (Statham P23BB), allowing measurement of ventilation rate (f_G, beats min^{-1}) and opercular pressure amplitude (P_{OP}, kPA). Opercular pressure amplitude was used as an index of ventilatory effort. The saline-filled dorsal aortic cannula was attached to a pressure transducer (Statham P23Db) for measurement of heart rate (f_H, beats min^{-1}) and dorsal aortic blood pressure (P_{DA}, kPA). The output from both transducers was displayed on a pen recorder (Gould 8188-2202-XX). Air-breathing frequency (f_{AB}, breaths h^{-1}) was visible as large pressure excursions on the opercular trace, associated with changes in f_H and P_{DA}. Air breaths were all verified visually through a small hole in a screen separating experimenter and fish.

Ventilatory and cardiovascular responses to a variety of different stimuli were measured in sham-operated and denervated animals. Experiments on any one fish were performed over a 2 day period, with overnight recovery between days. No experiments were initiated until ventilatory and cardiovascular variables had remained stable for 30 min. Animals were exposed to the following treatments, assigned randomly.

Aquatic hypoxia. Using a three-way valve at the inflow of the Plexiglas chamber, animals were acutely exposed to hypoxic water [O_2 partial pressure (P_{O_2})=6.31±0.16 kPa] obtained by bubbling 100 % N_2 counter-current to water flowing through a stripping column. Fish were exposed for 15 min, and then normoxic flow was resumed. Immediately prior to exposure, and following 5 min of exposure, arterial blood samples (700 µl) were withdrawn anaerobically from the dorsal aortic cannula, *via* a three-way stopcock at the pressure transducer. Blood samples were replaced with an equal volume of heparinised saline. 500 µl of blood was immediately centrifuged, the plasma decanted and frozen in liquid nitrogen for subsequent analysis of plasma catecholamine levels. Arterial blood pH (pHa), P_{O_2} (Pa_{O_2}) and O_2 content (Ca_{O_2}) were all measured on the remainder of the blood sample (see below for analytical methods). Animals were allowed a minimum of 2 h recovery following hypoxic exposure.

External NaCN. Either 1 ml of saline (control) or 1 mg of sodium cyanide (NaCN), dissolved in 1 ml of saline (experimental), was given as a bolus injection into the buccal cavity *via* the buccal cannula.

Internal NaCN. Either 300 µl of heparinised saline (control) or 300 µg of NaCN, dissolved in 300 µl of saline (experimental), was delivered as a bolus injection *via* the dorsal aortic (DA) cannula.

Catecholamine infusion. 0.5 ml kg^{-1} saline (control), 0.5 ml kg^{-1} 10^{-5} mol l^{-1} noradrenaline hydrochloride (Sigma) in saline (experimental) or 0.5 ml kg^{-1}

10^{-5} mol l^{-1} adrenaline bitartrate (Sigma) in saline (experimental) was infused *via* the DA cannula.

In all treatments, control and experimental injections or infusions were performed in random order. Animals were given a minimum of 30 min to recover between injections.

Analytical methods

Oxygen consumption was calculated for both aquatic and aerial phases. Steady-state measurements of P_{O_2} of inflow and outflow water were made using a Radiometer PHM 72 acid–base analyser and Radiometer O_2 electrode thermostatted to 20 °C. Oxygen consumption in the aquatic phase was then calculated by the Fick equation. Measurements of the decline in P_{O_2} in the sealed forward airspace over a 3 h period were used to calculate $\dot{V}_{O_2}$ by air-breathing. Plexiglas chambers used for O_2 consumption were those used by Randall *et al.* (1981). In that study, it was demonstrated that there was no exchange of gases between aquatic and aerial phases within the chamber.

Arterial blood pH and Pa_{O_2} were measured using a Radiometer PHM 72 acid–base analyser and associated electrodes, thermostatted to 20 °C. Arterial blood O_2 content was measured on 30 μl blood samples using the method of Tucker (1967) and a Radiometer electrode thermostatted to 20 °C. Arterial plasma noradrenaline and adrenaline concentrations ([NA] and [A], respectively) were measured on alumina-extracted samples by high performance liquid chromatography with electrochemical detection, using a Waters plasma catecholamine reverse-phase column, Waters M460 electrochemical detector and Waters 510 solvent delivery pump (Waters/Millipore), as described by Woodward (1982), with peaks generated on a chart recorder. Catecholamine concentrations were calculated by integrating the area under the peaks with Sigmascan (Jandel Scientific) and an Olivetti M24 computer, and comparing with appropriate standards.

Data analysis

Ventilatory and cardiovascular responses were analysed for a control period and at designated intervals following each experimental intervention. Ventilation and heart rate were counted for 30 s in each minute, and P_{OP} and P_{DA} were averaged from six measurements within that period; for 2 min control and at 1, 2.5, 5, 10 and 15 min following intervention. Air-breathing frequency was averaged for the whole measurement period following intervention. When a blood sample was withdrawn, cardiovascular measurements were made at 4 min.

Cardiovascular and gill ventilatory responses were normalised as percentage change from control and, following arcsine transformation, compared at each time interval with an analysis of variance (ANOVA). In those cases where the ANOVA indicated a significant difference amongst time intervals, each time interval was compared *a posteriori* with the control value. Gill ventilatory responses to NA and A infusion were also assessed by comparing control and peak responses with a

paired *t*-test, using the normalised transformed values. Normalised responses were used for graphical display.

Blood measurements taken during aquatic hypoxia exposure were compared with measurements obtained immediately prior to hypoxia exposure with a paired *t*-test. Air-breathing frequency under control normoxic conditions was compared with f_{AB} under hypoxic conditions using a paired *t*-test. For NaCN and catecholamine infusions, f_{AB} following control (saline) injection was compared with f_{AB} following the associated experimental injection using paired *t*-tests. Air-breathing frequency was compared between sham-operated and denervated fish, for each experimental intervention, using unpaired *t*-tests. $P<0.05$ was taken as the fiducial limit of significance.

Results

Resting variables

Gill denervation and pseudobranch ablation resulted in a significant reduction in $\dot{V}_{O_2}$, f_{AB} and Pa_{O_2} and a significant increase in the level of circulating NA. At 20°C, in aquatic normoxia, sham-operated *Amia* obtained 0.1 % of their total O_2 uptake by air-breathing. Following gill denervation and pseudobranch ablation, animals (denervates) had a significantly reduced O_2 consumption rate (30 % lower than sham-operated *Amia*), and there was no O_2 uptake by air-breathing (Table 1). Mean control values for P_{DA}, f_H, P_{OP} and f_G are also given in Table 1. There was no significant difference between sham-operated *Amia* and denervates for these variables, although denervates appeared to have a higher P_{OP}. During normoxia, f_{AB} was very low in the sham-operated *Amia*, usually zero, and only one denervate breathed air, on two occasions (Table 2). In normoxia, denervates showed no differences in pHa, Ca_{O_2} and [A] as compared with sham-operated *Amia*, but Pa_{O_2} was significantly lower and [NA] significantly higher in denervates (Table 3).

Table 1. *Normoxic values of* $\dot{V}_{O_2}$, P_{DA}, f_H, P_{OP} *and* f_G

	Sham-operated *Amia*	Denervated *Amia*
$\dot{V}_{O_2}$(t) (mg O_2 kg^{-1} h^{-1})	52.8±4.9	36.7±3.3*
$\dot{V}_{O_2}$(a) (mg O_2 kg^{-1} h^{-1})	0.06±0.02	0*
$\dot{V}_{O_2}$(w) (mg O_2 kg^{-1} h^{-1})	52.7±4.9	36.6±3.3*
P_{DA} (kPa)	2.88±0.01	2.98±0.006
f_H (beats min^{-1})	30.0±0.11	25.6±0.04
P_{OP} (kPa)	0.066±0.0001	0.156±0.001
f_G (beats min^{-1})	12.2±0.09	10.7±0.05

Values are means±S.E.; * denotes significantly different ($P=0.05$) from sham-operated; $N=6$ for sham $\dot{V}_{O_2}$; $N=7$ for denervate $\dot{V}_{O_2}$.

Cardiovascular and ventilatory variables are the mean of 48 measurements on six sham-operated *Amia* and 74 measurements on seven denervates.

$\dot{V}_{O_2}$, oxygen consumption, (t) total, (a) air-breathing, (w) water-breathing; P_{DA}, dorsal aortic blood pressure; P_{OP}, opercular pressure amplitude; f_H, heart rate; f_G, gill ventilation rate.

Effects of aquatic hypoxia

In sham-operated *Amia*, acute exposure to aquatic hypoxia elicited significant cardiovascular and ventilatory responses (Fig. 1). A gradually developing bradycardia was evident, with f_H significantly reduced following 15 min of exposure. Gill ventilation increased, with significant changes in P_{OP} and f_G at 5 min that were sustained until the end of hypoxic exposure. Air-breathing frequency increased significantly (Table 2), with most of the air-breaths occurring in the first 5 min of hypoxic exposure. At 5 min of exposure, pHa and Ca_{O_2} were maintained at pre-exposure values, but Pa_{O_2} decreased significantly. Arterial NA concentrations increased, but there was no significant effect on [A] (Table 3).

In denervates, the response to aquatic hypoxia was distinctly different (Fig. 1). The bradycardia response was abolished. Gill ventilation did not increase until 10 min of exposure, with a sustained increase in f_G and a transient increase in P_{OP} that was no longer evident at 15 min. There was no change in f_{AB} during hypoxia, and air-breathing responses were abolished (Table 2). At 5 min of exposure, pHa was not changed from pre-exposure values. Arterial blood O_2 content was not maintained at the pre-exposure value but decreased significantly, as did Pa_{O_2}. The

Table 2. *Air-breathing frequency (breaths h^{-1})*

	Sham-operated *Amia*	Denervated *Amia*	Partially denervated *Amia*
Hypoxia	5.1±1.5	0†	–
External saline	0	0.7±0.7	0
External NaCN	1.33±0.9	0.7±0.7	4.0±1.3*
Internal saline	0.7±0.7	0	–
Internal NaCN	0	0	–
Noradrenaline infusion	0	0	–
Adrenaline infusion	0	0	–

Values are mean±S.E.; †denotes significantly different from sham-operated, hypoxia (P=0.05); *denotes significantly different (P=0.05) from sham-operated, saline injection.
N=6 for sham-operated *Amia*, 7 for denervates, and 7 for partial denervates.

Table 3. *Arterial pH, blood gases and noradrenaline and adrenaline concentrations*

	pHa	Pa_{O_2} (kPa)	Ca_{O_2} (kPa)	[Noradrenaline] (nmol l^{-1})	[Adrenaline] (nmol l^{-1})
Sham-operated normoxia	7.72±0.02	6.2±1.6	0.59±0.1	25.3±3.7	5.2±3.0
Sham-operated hypoxia	7.73±0.03	3.7±0.6*	0.41±0.05	42.6±5.9*	7.8±3.1
Denervated normoxia	7.75±0.02	2.2±0.2†	0.51±0.09	90.0±17.3†	18.9±8.5
Denervated hypoxia	7.72±0.02	1.5±0.2*	0.23±0.05*	132.7±25.6*	86.4±38.5

Values are mean±S.E.; *denotes significantly different from normoxic control; †denotes significantly different from sham-operated normoxic control.
N=6 for both sham-operated *Amia* and denervates.

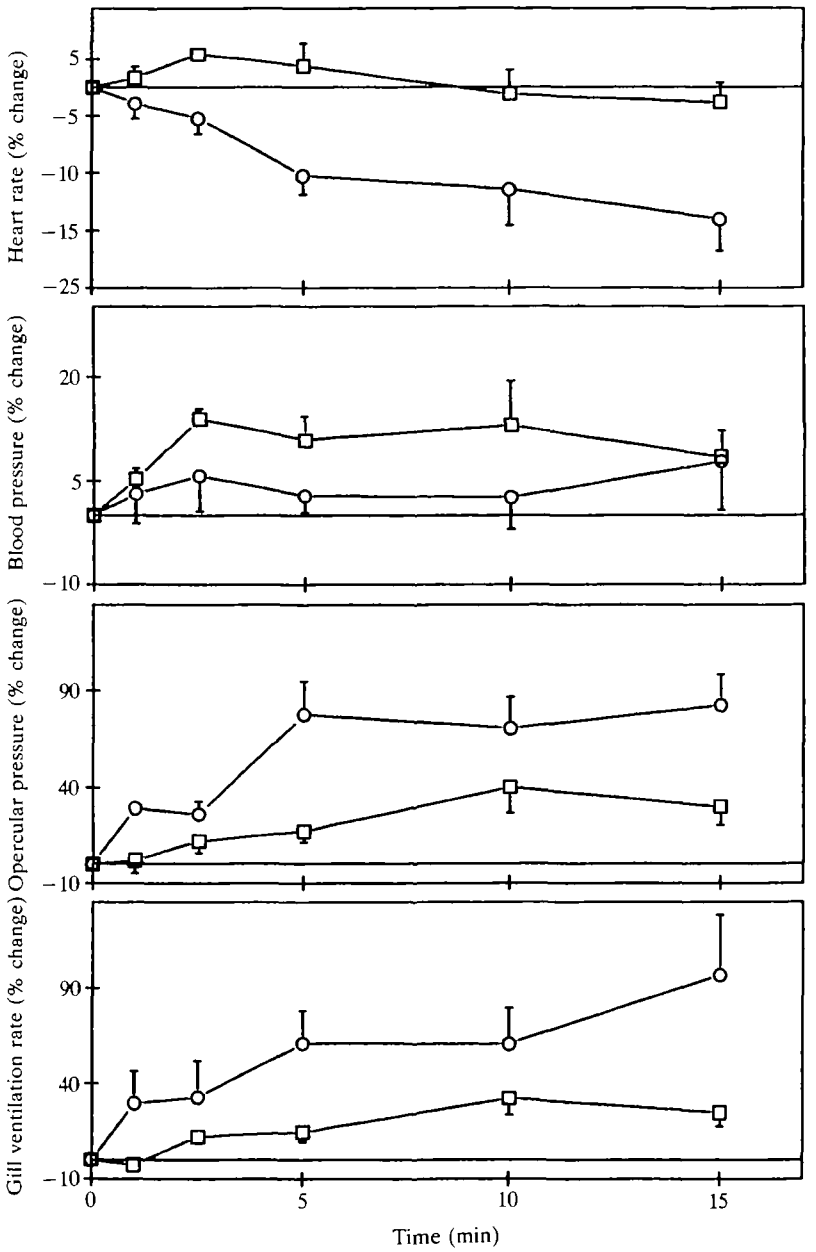

Fig. 1. Average response (normalised as mean percentage change from control, ±1 S.E.M.) of dorsal aortic peak systolic blood pressure (P_{DA}), heart rate (f_H), opercular pressure amplitude (P_{OP}) and gill ventilation rate (f_G) in sham-operated (○) and denervated (□) *Amia* during 15 min of exposure to aquatic hypoxia (P_{O_2}=6.31±0.16 kPa). N=6.

concentration of circulating NA increased, but there was no significant effect on [A] (Table 3).

Effects of NaCN

In sham-operated *Amia*, bolus injection of NaCN into the buccal cavity (Fig. 2) elicited a transient bradycardia and a transient increase in P_{OP}. Arterial blood pressure and f_G did not change significantly from control values. In two out of six fish, external NaCN immediately stimulated an air-breath (Table 2) but, overall, NaCN in the buccal cavity had no significant effect on air-breathing. It is of interest to note that partial denervates (i.e. animals with a branchial branch of IX or X and/or the pseudobranch intact) showed an immediate and significant increase in f_{AB} within 5 min following external NaCN (Table 2). External saline control injections had no effect on any variable (Fig. 2).

In denervates (Fig. 2), all cardiovascular and ventilatory responses to NaCN in the buccal cavity were abolished, with no significant change in any variable over time. One animal took an air-breath in response to both external saline and NaCN, but in the latter case at 13 min post-injection, which indicates that it was not in response to the NaCN (Table 2). Injection of a saline bolus into the buccal cavity had no effect on any variable (Fig. 2).

Bolus injection of NaCN into the dorsal aorta of sham-operated *Amia* had no significant effect on P_{DA} and f_H, but significantly stimulated P_{OP} and f_G (Fig. 3). P_{OP} increased transiently at 2.5 min post-injection, and f_G was elevated at 1 and 2.5 min. Internal NaCN had no effect on f_{AB} (Table 2), and injection of a saline bolus into the DA had no effect on any variable (Fig. 3).

In denervates (Fig. 3) the ventilatory responses to internal NaCN were abolished, with no significant changes in P_{OP} or f_G. Cardiovascular variables showed a similar trend to those of sham-operated *Amia*; the changes over time were not statistically significant. There was no air-breathing response to internal NaCN in the denervates. Injection of a saline bolus had no effect on any variable (Fig. 3). Fig. 4 shows representative traces of cardiovascular and ventilatory responses to NaCN in sham-operated *Amia* and denervates.

Effects of catecholamines

In sham-operated *Amia*, NA infusion (Fig. 5) significantly increased P_{DA}, f_H and P_{OP}. There was no significant effect on f_G, and no stimulation of f_{AB}. Arterial blood pressure showed a transient increase at 2.5 min and f_H was elevated at 2.5, 5 and 10 min following infusion. Opercular pressure amplitude was significantly elevated at 2.5 min post-infusion, and remained elevated throughout the remainder of the measurement period. Adrenaline infusion had similar effects to NA infusion on cardiovascular variables (Fig. 5), significantly increasing P_{DA} from 1 min post-infusion until the end of the measurement period, but only transiently stimulating f_H, at 2.5 min. There was no statistically significant stimulation of P_{OP} or f_G by A, as assessed by ANOVA. However, a paired *t*-test showed a significant increase in mean P_{OP} and f_G at 2.5 min post-infusion. There was no stimulation of

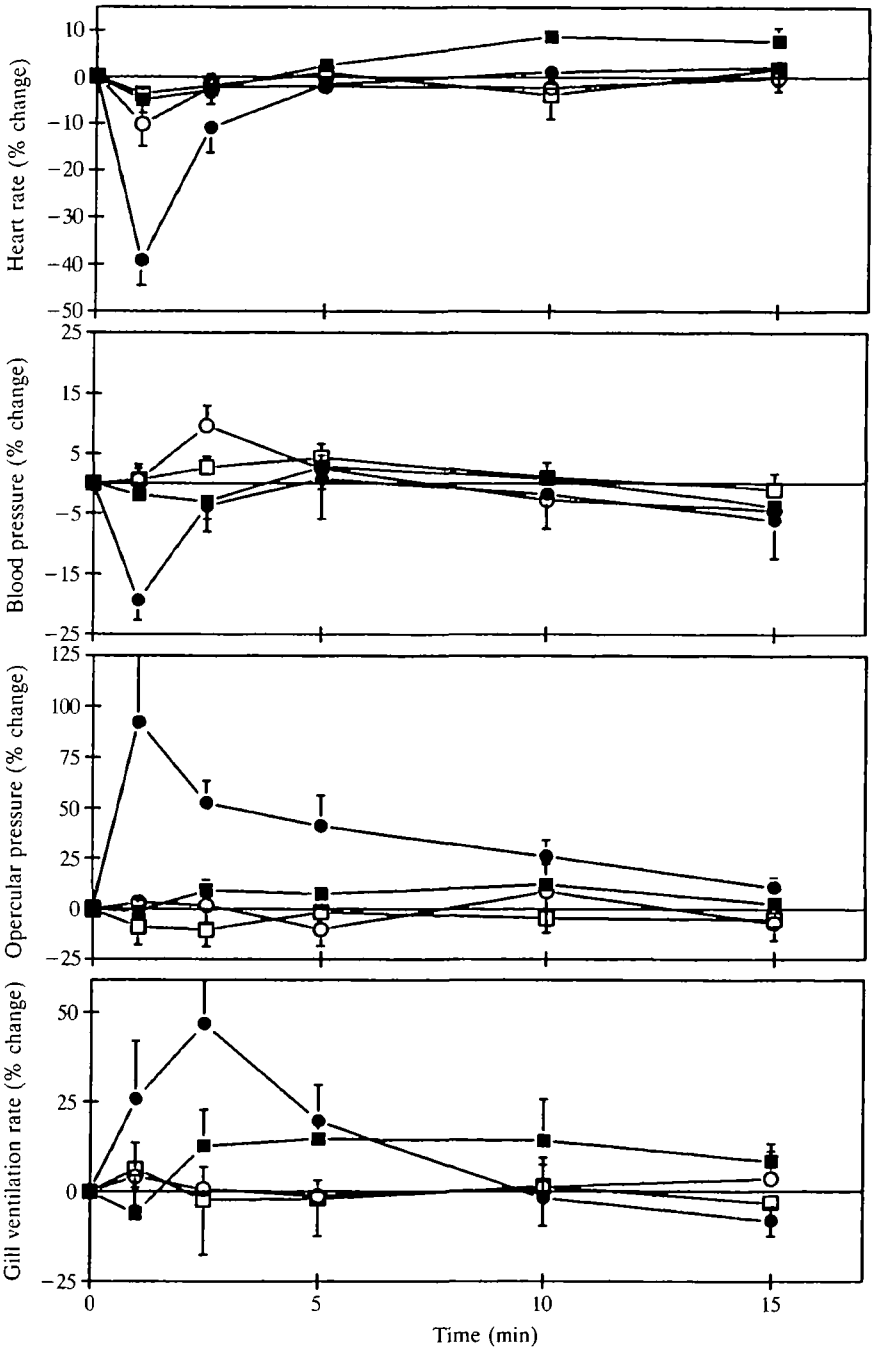

Fig. 2. Average response (normalised as mean percentage change from control, ±1 S.E.M.) of dorsal aortic peak systolic blood pressure (P_{DA}), heart rate (f_H), opercular pressure amplitude (P_{OP}) and gill ventilation rate (f_G) in sham-operated (circles) and denervated (squares) *Amia* following injection of 1 mg of NaCN (filled symbols) or saline (open symbols) into the buccal cavity. $N=6$ for sham-operated; $N=7$ for denervates.

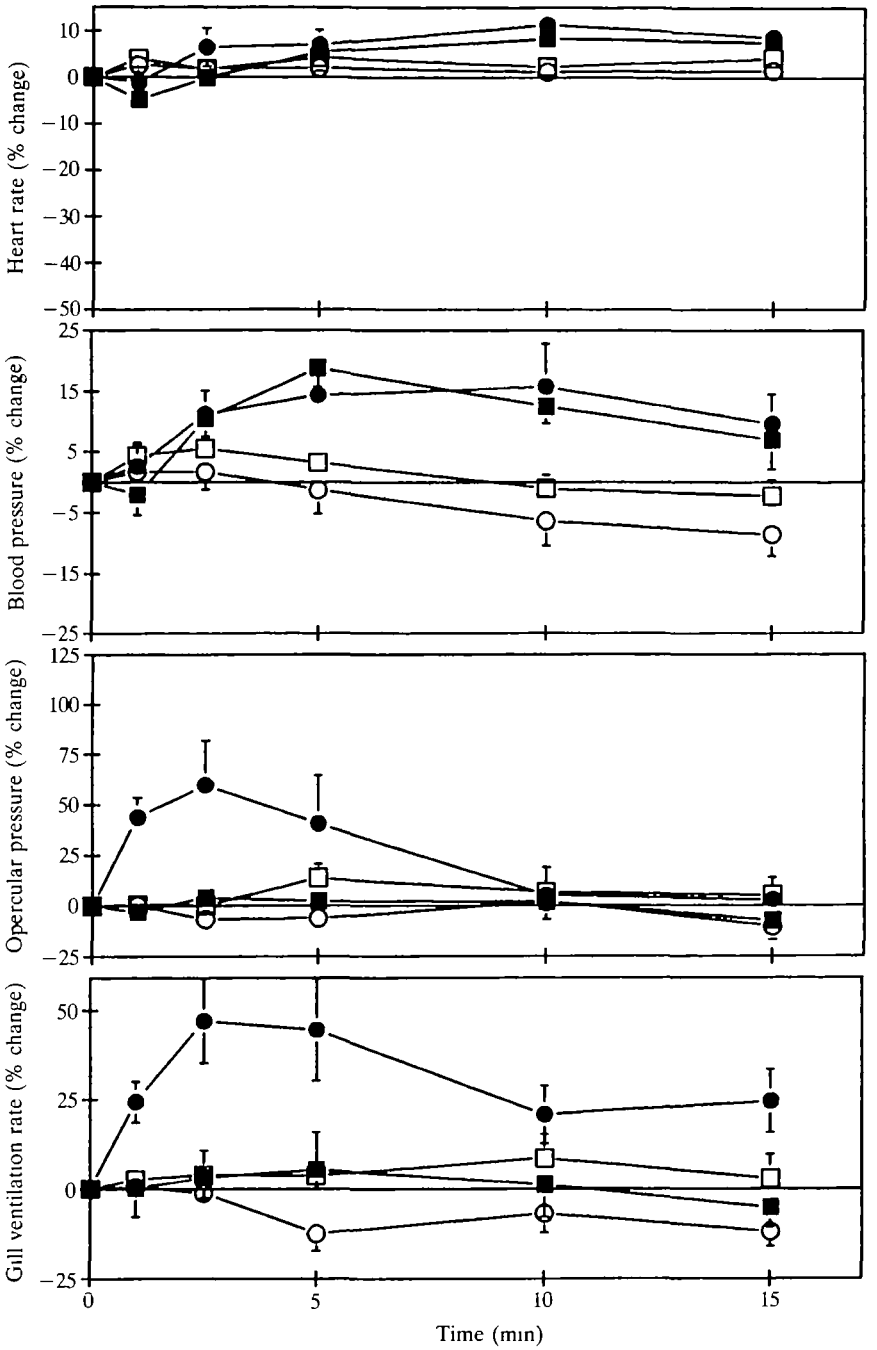

Fig. 3. Average response (normalised as mean percentage change from control, ±1 S.E.M.) of dorsal aortic peak systolic blood pressure (P_{DA}), heart rate (f_H), opercular pressure amplitude (P_{OP}) and gill ventilation rate (f_G) in sham-operated (circles) and denervated (squares) *Amia* following injection of 300 μg of NaCN (filled symbols) or saline (open symbols) into the dorsal aorta. $N=6$ for sham-operated; $N=7$ for denervates.

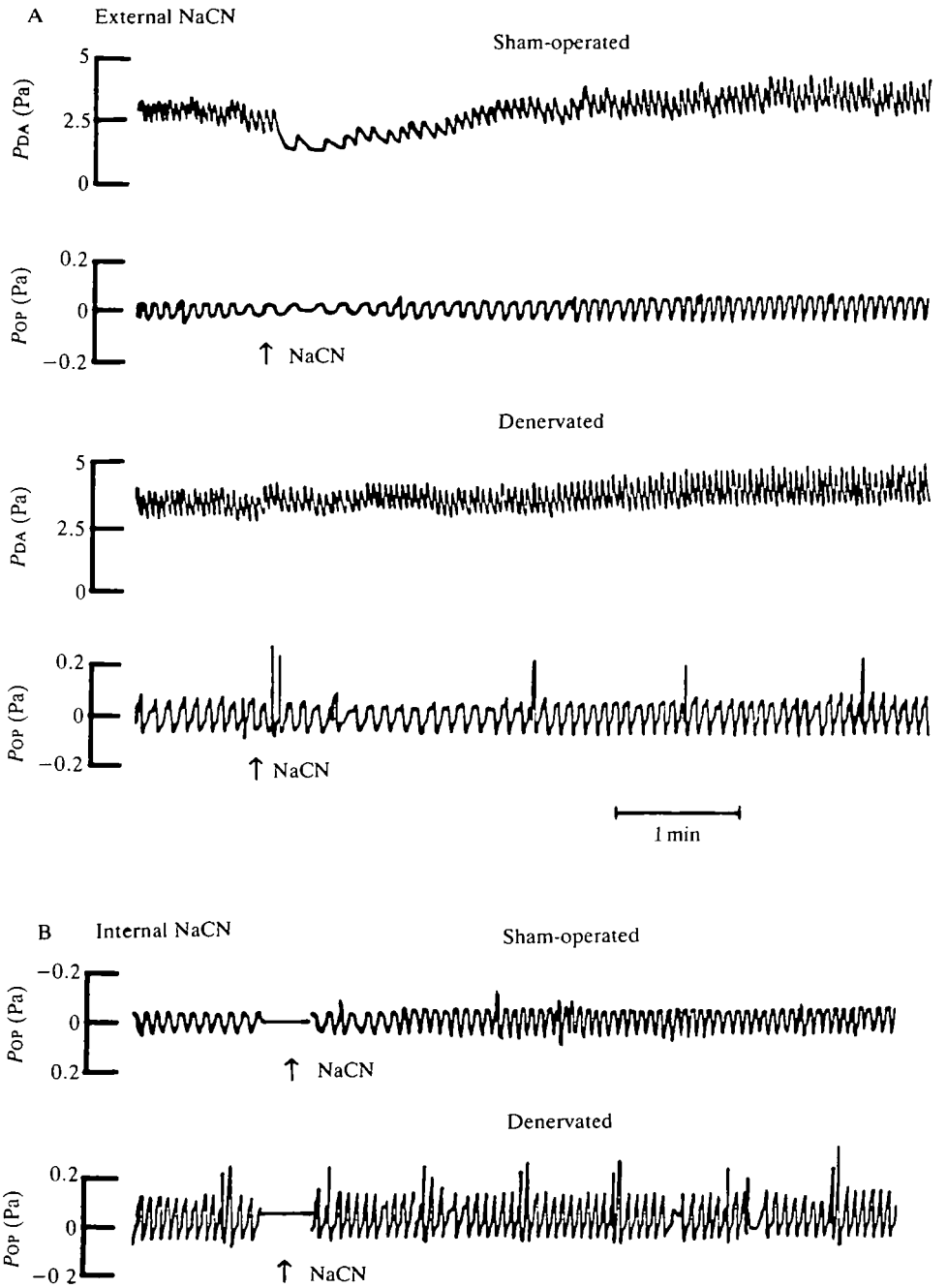

Fig. 4. Representative traces of cardiovascular and gill ventilatory responses to external (A) and internal (B) NaCN in sham-operated and denervated *Amia*.

f_{AB} (Table 2). Control saline infusion had no effect on P_{DA}, f_H, P_{OP}, f_G or f_{AB} (Fig. 5).

In denervated fish (Fig. 5), NA had effects on P_{DA} and f_H very similar to those seen in sham-operated *Amia*, but there was no statistically significant effect on P_{OP} or f_G, when measured by ANOVA. Adrenaline also stimulated P_{DA} and f_H in a manner similar to the response of the sham-operated fish. There was no significant effect on P_{OP} following A infusion, but f_G increased at 2.5, 5 and 10 min post-infusion, when measured by ANOVA. Whilst NA and A showed no statistically significant effect on P_{OP} as measured by ANOVA, a comparison of denervate mean control P_{OP} and f_G with mean P_{OP} and f_G at 2.5 min post-infusion, by paired *t*-test, shows a significant increase in both ventilatory variables at 2.5 min (this was not the case for NaCN exposure in denervates). Saline control infusion had no effect on any measured variable (Fig. 5).

Discussion

The present study demonstrates that in *Amia*, section of all branchial branches of cranial nerves IX and X and extirpation of the pseudobranch abolish air-breathing and cardiac reflex responses to hypoxia and NaCN. Gill ventilatory responses to NaCN are abolished. Gill ventilatory responses to hypoxia and catecholamines are significantly attenuated. These results indicate that air-breathing and cardiac reflex responses to hypoxia are mediated by O_2-sensitive chemoreceptors in the gills and pseudobranch, innervated by cranial nerves VII, IX and X, but that gill ventilatory reflex responses are mediated to some extent *via* an extrabranchial pathway.

Resting cardiovascular, ventilatory and blood-gas variables

Gill denervation did not change any resting cardiovascular or gill ventilatory variables significantly. This indicates that the motor pathways to the buccal and opercular pumps were not damaged by surgery, and also that *Amia* is different from *L. oculatus*, where section of the branchial branches of cranial nerve IX significantly attenuates resting P_{OP} and f_G (Smatresk, 1991). A reduction or abolition of afferent information about water and blood O_2 levels and abolition of all efferent vascular and postural motor control of the gill arches probably combined to result in the measured reduction in Pa_{O_2} and $\dot{V}_{O_2}$ seen in denervated *Amia* as compared with sham-operated fish. The denervates, however, had similar Ca_{O_2} and pHa values to sham-operated *Amia*, indicating that the former were not hypoxaemic and were not accumulating lactic acid as a result of anaerobic metabolism. Elevated circulating NA levels in denervated *Amia* indicate that they were more stressed than sham-operated *Amia*, as catecholamines are released into the circulation in response to stress in fish (Nakano and Tomlinson, 1967).

Sham-treated *Amia* did not breathe air as much during aquatic normoxia as noted by Johansen *et al.* (1970) and Randall *et al.* (1981). Denervated animals did not breathe air at all, except for one individual that did so on two occasions within

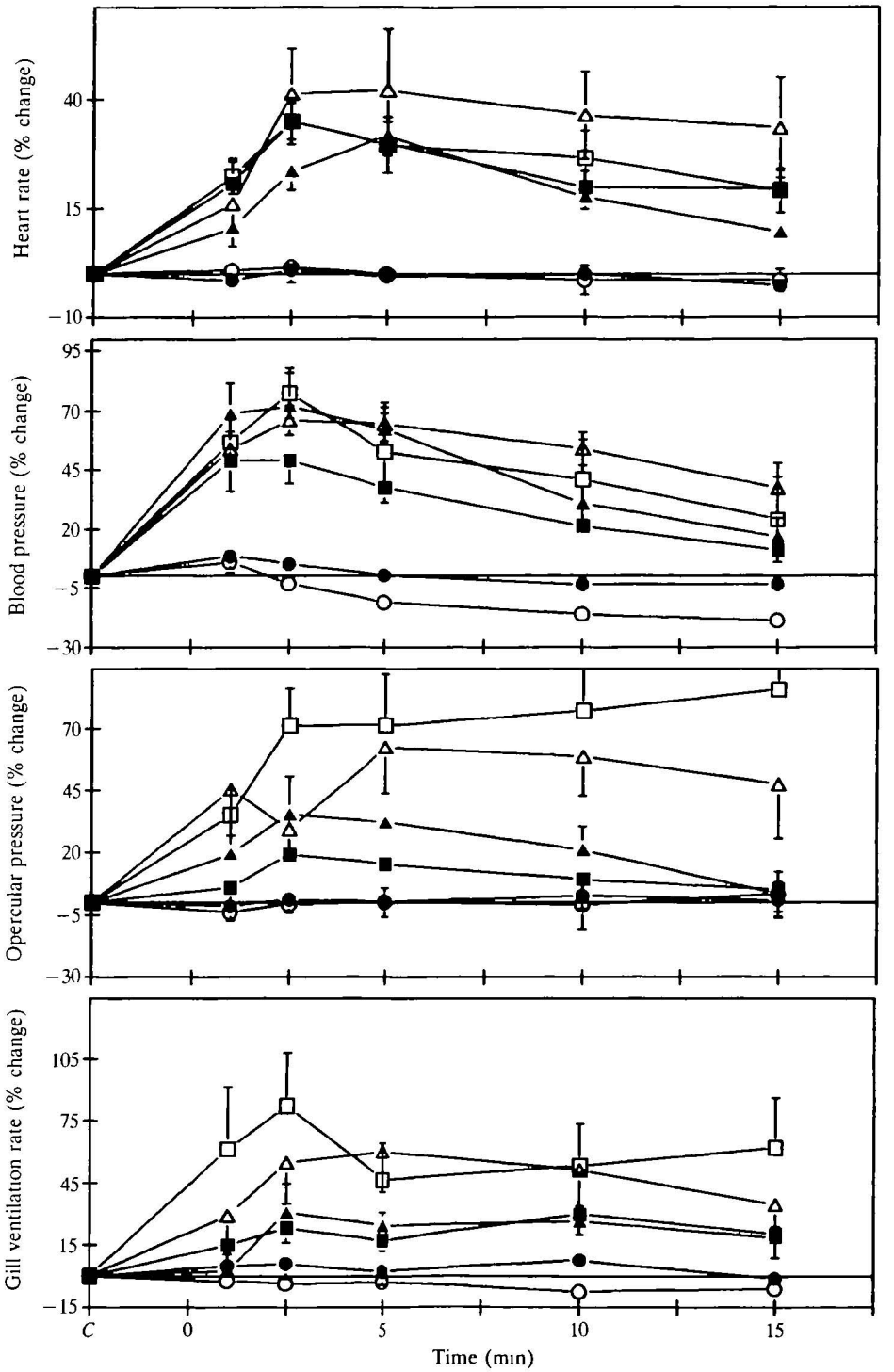
Heart rate (% change)
40
15
−10
Blood pressure (% change)
95
70
45
20
−5
−30
Opercular pressure (% change)
70
45
20
−5
−30
Gill ventilation rate (% change)
105
75
45
15
−15
C
0
5
10
15
Time (min)

Fig. 5

Fig. 5. Average response (normalised as mean percentage change from control, ±1 S.E.M.) of dorsal aortic peak systolic blood pressure (P_{DA}), heart rate (f_H), opercular pressure amplitude (P_{OP}) and gill ventilation rate (f_G) in sham-operated (open symbols) and denervated (filled symbols) *Amia* following infusion of 1 ml kg^{-1} of adrenaline (triangles) or noradrenaline (squares) into the dorsal aorta. N=6, *C*, control.

1 h. This is similar to *L. oculatus*, where complete branchial denervation severely reduced resting f_{AB} (Smatresk, 1991). This indicates that air-breathing behaviour in both *Amia* and *L. oculatus* is mainly, but not entirely, dependent on afferent input from the gills.

Effects of denervation on cardiovascular responses

Abolition of the f_H response to hypoxia and external NaCN following gill denervation indicates that reflex bradycardia in *Amia* is controlled by O_2-sensitive chemoreceptors in the gills, as is the case in exclusively water-breathing teleosts, e.g. trout (Smith and Jones, 1978), cod (Fritsche and Nilsson, 1989) and channel catfish (Burleson and Smatresk, 1990*a*), where bradycardia is abolished by section of cranial nerves IX and X to the gills.

In sham-operated *Amia*, stimulation of a bradycardia by NaCN given in the buccal cavity, but not by NaCN infused into the dorsal aorta, supports previous studies that indicate that O_2-sensitive chemoreceptors mediating bradycardia are externally oriented (Saunders and Sutterlin, 1971; Burleson and Smatresk, 1990*b*). Cardiovascular responses by *Amia* to external and internal NaCN are similar to the responses of channel catfish (Burleson and Smatresk, 1990*a*,*b*) but contrast with the responses of the air-breathing fish *Lepisosteus osseus* (Smatresk, 1986; Smatresk *et al.* 1986). In *L. osseus*, heart rate is either not changed by external or internal NaCN (Smatresk, 1986) or internal NaCN produces a bradycardia (Smatresk *et al.* 1986).

In sham-operated *Amia* and denervates, stimulation of P_{DA} and f_H by catecholamines is probably a result of direct effects on the myocardium and peripheral vasculature (Wood and Shelton, 1980; Farrell *et al.* 1986).

Effects of denervation on ventilatory responses

Increases in P_{OP}, f_G and f_{AB} following hypoxic exposure in sham-operated *Amia* were similar to the responses of most air-breathing fish (Smatresk, 1988). Denervates did not air-breathe during hypoxia, providing evidence that O_2-sensitive chemoreceptors stimulating this behaviour are located in the gills or pseudobranch. This is similar to the African lungfish *Protopterus aethiopicus* (Lahiri *et al.* 1970), where partial gill denervation attenuated the air-breathing response to hypoxia, and to *L. oculatus*, where complete branchial denervation abolished air-breathing and gill ventilatory responses to hypoxia (Smatresk, 1991).

Sham-operated *Amia* required large doses of NaCN to elicit ventilatory responses, as compared with *L. osseus* (Smatresk, 1986). In *L. osseus*, external

NaCN stimulated air-breathing but had no significant effect on gill ventilation (Smatresk, 1986), whereas in *Amia* there was an increase in gill ventilation but no significant effect on f_{AB}. *L. osseus* have reduced gills compared with purely water-breathing fish (Smatresk and Cameron, 1982) but *Amia* do not (Daxboeck *et al.* 1981). These facts suggest that under natural conditions *Amia* relies on air-breathing for O_2 uptake less than does *L. osseus*, and demonstrate the variation in air-breathing strategies amongst fish.

Injections of NaCN into the dorsal aorta of sham-operated *Amia* stimulated a similar response to that seen in *L. osseus* (Smatresk, 1986), where internal NaCN significantly stimulated gill ventilation but not air-breathing. This is unlike the response of *P. aethiopicus*, where internally administered NaCN stimulated air-breathing (Lahiri *et al.* 1970). It is unknown whether internal and external NaCN injections stimulated the same or different groups of ventilatory reflex receptors in *Amia*.

It is interesting that hypoxia significantly stimulated air-breathing in sham-operated *Amia* but external and internal NaCN did not. It is possible, however, that two groups of O_2-sensitive receptors, oriented externally and internally, exist in *Amia* and that information from both receptor groups is integrated to produce a final air-breathing pattern. This is known to be the case in *L. osseus*, where internally oriented receptors set the level of hypoxic drive and externally oriented receptors set the balance of air-breathing *vs* gill ventilation (Smatresk *et al.* 1986). In *Amia*, during hypoxia, both groups may have been stimulated, leading to air-breathing. External or internal NaCN only stimulated one group of receptors, and thus only sometimes stimulated air-breathing. In incomplete denervates (i.e. animals with a branchial nerve and/or pseudobranch intact) external NaCN consistently stimulated air-breathing. Partial denervation may have affected the balance of information from both groups and led to more frequent air-breaths.

The complete abolition of all ventilatory responses to NaCN following branchial denervation and pseudobranch ablation clearly indicates that these reflexes are mediated by O_2-sensitive chemoreceptors situated in the gills and pseudobranch, innervated by cranial nerves VII, IX and X, and that following denervation *Amia* do not retain O_2-sensitive-chemoreceptor-mediated ventilatory reflexes. Thus, the P_{OP} and f_G responses seen in hypoxic denervates are not mediated by an extrabranchial O_2-chemoreceptor (Bamford, 1974).

Infusion of catecholamines into sham-operated *Amia* produced ventilatory effects similar to those seen in intact fish, with increases in P_{OP} and f_G but no change in f_{AB} (McKenzie *et al.* 1991). Following denervation, the ventilatory response to catecholamines was attenuated. Denervation of aortic and carotid bodies in mammals causes a reduction in ventilatory sensitivity to catecholamine infusion (Dempsey *et al.* 1986), suggesting that, in *Amia*, stimulation of gill ventilation by catecholamines may be largely *via* stimulation of O_2-sensitive chemoreceptors in the gills. It is also possible, however, that the elevated endogenous NA levels in denervated animals reduced ventilatory sensitivity to exogenous catecholamine infusion. The existence, however, of a ventilatory

response to catecholamine infusion in denervates clearly indicates that catecholamines stimulate ventilation *via* an extrabranchial pathway, possibly centrally. Catecholamines can cross the blood–brain barrier in fish (Nekvasil and Olson, 1986) and micro-injection of NA into the fourth ventricle stimulates 'fictive' ventilation in curarised dogfish (Taylor and Randall, 1988). It has been suggested that circulating catecholamines mediate ventilatory responses to hypoxia in trout and dogfish (Randall and Taylor, 1989; Taylor and Randall, 1988; Aota *et al.* 1990). The absence of an air-breathing response to catecholamine infusion in intact and denervated *Amia* indicates that catecholamines stimulate ventilation at a site, be it central or peripheral, that is not involved in controlling air-breathing. This suggests that some sites controlling reflex gill ventilatory responses are spatially and/or pharmacologically separate from sites controlling air-breathing reflexes.

Both sham-operated *Amia* and denervates showed a significant increase in [NA] following hypoxic exposure, which supports the view that circulating catecholamines are involved in ventilatory control. The complete abolition of all ventilatory responses to hypoxia following branchial denervation in anaesthetised, spontaneously breathing *I. punctatus* (Burleson and Smatresk, 1990*a*) and *L. oculatus* (Smatresk, 1991) may have occurred because these species are less sensitive to circulating NA than is *Amia*, or because the use of an anaesthetised preparation compromised ventilatory responses to catecholamines.

In conclusion, the removal of afferent information from the gills reduces resting $\dot{V}_{O_2}$ in *Amia*. *Amia* possess two distinct groups of branchial O_2-sensitive chemoreceptors. Reflex bradycardia is controlled by an externally oriented O_2-sensitive chemoreceptor group, whereas ventilatory reflexes are mediated by both internal and external O_2-sensitive receptors, or by a group sensitive to both internal and external conditions. Reflex air-breathing responses to hypoxia are mediated exclusively by O_2-sensitive chemoreceptors in the gills, and *Amia* possess no extrabranchial O_2-sensitive-chemoreceptor-mediated ventilatory reflexes. The evidence indicates that in denervated animals gill ventilatory responses to hypoxia may be mediated centrally by circulating catecholamines.

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