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EFFECTS OF SECTIONING CRANIAL NERVES IX AND X ON CARDIOVASCULAR AND VENTILATORY REFLEX RESPONSES TO HYPOXIA AND NaCN IN CHANNEL CATFISH

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Summary

The afferent pathways mediating cardiovascular and ventilatory hypoxic reflexes were identified in anaesthetized, spontaneously breathing (ASB) channel catfish, *Ictalurus punctatus* (Rafinesque), by bilateral section of cranial nerve IX and branchial branches of cranial nerve X to the four gill arches (Xb_1–Xb_4). Cardiovascular and ventilatory responses to hypoxia and NaCN were attenuated by partial denervation of the gills. There were no significant cardiovascular or ventilatory reflex responses to either hypoxia or NaCN after total branchial denervation. This suggests that hypoxic reflexes in channel catfish are mediated by O_2-sensitive chemoreceptors located peripherally in the gills and innervated by cranial nerves IX and X.

Introduction

The reflex responses to hypoxia in teleost fishes appear to be mediated by chemoreceptors that are sensitive to external (water) and internal (blood or tissue) oxygen levels. Hypoxic bradycardia results from stimulation of externally oriented branchial chemoreceptors (Saunders and Sutterlin, 1971; Smith and Jones, 1978; Daxboeck and Holeton, 1978; Burleson and Smatresk, 1990). Ventilatory responses to hypoxia, however, may be elicited by low O_2 tensions in either blood or water (e.g. Saunders and Sutterlin, 1971; Burleson and Smatresk, 1990). Hypoxic bradycardia in salmonids (Smith and Jones, 1978; Daxboeck and Holeton, 1978) and Atlantic cod *Gadus morhua* Linnaeus (Fritsche and Nilsson, 1989) is mediated by O_2-sensitive chemoreceptors in the first gill arch, innervated by cranial nerves IX and X. Chemoafferent activity responding to internal and external hypoxia has been recorded from the first gill arch of yellowfin tuna *Thunnus albacares* (Bonnaterre) (Milsom and Brill, 1986), but the chemoreceptor loci and afferent pathways involved in ventilatory control have not been unequivocally identified

Key words: *Ictalurus punctatus*, gill, glossopharyngeal nerve, vagus nerve, O_2-sensitive chemoreceptors, hypoxia, NaCN.

for any fish. Sectioning various combinations of branchial nerves IX and X has failed to abolish ventilatory responses to hypoxia in tench, *Tinca tinca* (Linnaeus) (Shelton, 1959; Hughes and Shelton, 1962), sea raven, *Hemitripterus americanus* (Gmelin) (Saunders and Sutterlin, 1971) and salmonids (Smith and Jones, 1978; Smith and Davie, 1984), leading to suggestions of extra-branchial chemoreceptor loci (Bamford, 1974; Jones, 1983). Alternatively, chemoreceptors controlling the ventilatory response to hypoxia may be widely distributed throughout the gills, and only complete branchial deafferentation may abolish hypoxic reflexes.

In mammals, O_2-sensitive chemoreceptors are associated with the carotid and aortic arches and are innervated by branches of IX and X. The arterial arches in mammals are derived from the phylogenetically ancient gill arches of the hypothetical ancestor of all vertebrates. Given this, the most likely location for O_2-sensitive chemoreceptors in fish would be the gills. The branchial innervation of fish consists of branches of cranial nerves VII, IX and X (Nilsson, 1984). The pseudobranch is innervated by branches of VII and IX (Laurent and Dunel-Erb, 1984). The first gill arch receives innervation from cranial nerves IX and X and the remaining arches are innervated by branchial branches of nerve X (Nilsson, 1984). The purpose of this study was to test the hypothesis that a diffuse set of branchial chemoreceptors mediates the reflex responses to hypoxia, by studying the effects of partial and complete branchial denervation on the ventilatory and cardiovascular responses to hypoxia and NaCN in anaesthetized spontaneously breathing (ASB) catfish. Channel catfish were chosen because they lack a pseudobranch, eliminating the need to section cranial nerve VII and simplifying denervation. The ASB preparation has previously been useful for studying chemoreflex responses and localizing chemoreceptors in longnose gar, *Lepisosteus osseus* (Linnaeus) and catfish (Smatresk *et al.* 1986; Burleson and Smatresk, 1990), because it eliminates a number of difficulties associated with branchial denervation in conscious fish (Burleson and Smatresk, 1989).

Materials and methods

Channel catfish were obtained from a commercial supplier. They were maintained in 500 l tanks equipped with recirculating gravel filters at holding temperatures between 20 and 25 °C, on 12 h/12 h light/dark cycles, and were fed commercial catfish pellets.

Animal preparation

Fish were anaesthetized in MS-222 (ethyl *m*-aminobenzoate) dissolved in oxygenated, dechlorinated tap water (0.01 %), transferred to a surgery table and artificially ventilated with the anaesthetic solution. Owing to the hardness of the local water, buffering the anaesthetic water was not necessary. The dorsal aorta (DA) was cannulated (Intramedic PE-50) as described by Burleson and Smatresk (1989). An additional cannula (Intramedic PE-160) was inserted into the opercular chamber to record opercular pressure. After cannulation, the branchial nerves

were exposed by reflecting the operculum anteriorly and making an incision in the epithelium dorsal and posterior to the gill filaments. A wet tissue protected the delicate gill filaments and prevented desiccation. Since only half the gills were irrigated during this exposure, the water was bubbled with oxygen to help maintain arterial P_{O_2}. The nerves were gently teased from the connective tissue, avoiding damage to the gill efferent vasculature, and sectioned with iris scissors central to the point at which the nerve divides into pre- and post-trematic branches. The procedure was then repeated on the other side, so that nerves were sectioned bilaterally. Sham operations involved identical nerve manipulation but no nerve section. The ventilatory muscles and branchial vasculature were not damaged by this procedure. A progressive series of bilateral denervations was used to assess the role of chemoreceptors in various gill arches on cardiovascular and ventilatory reflexes. The following nerve section groups were used for the hypoxia experiments: no nerves sectioned (sham control, N=7); IX only (N=8); IX+Xb_1 (N=6); IX+Xb_{1-3} (N=5); IX+Xb_{1-4} (complete denervation, N=7). Identical experiments were also performed on a group of intact, atropinized fish (N=5). For NaCN experiments the following nerve sections were used: sham control (N=8); IX+Xb_1 (N=6); Xb_{1-3} (N=4); IX+Xb_{1-3} (N=5); IX+Xb_{1-4} (N=8) and intact, atropinized fish (N=5). Success of nerve sections was confirmed *post mortem*.

After surgery, fish were transferred to an acrylic holding chamber where ventilation was assisted with a low flow of water containing a reduced amount of MS-222 (0.004 %). At this concentration of MS-222 the fish ventilated spontaneously and exhibited similar hypoxic reflexes to conscious, unanaesthetized catfish (Burleson and Smatresk, 1989). Assisting gill ventilation with a low flow of water past the gills does not interfere with spontaneous ventilatory movements, but does help to maintain approximately normal DA O_2 tensions in this preparation (Burleson and Smatresk, 1989). The flow rate for the assisted ventilation was maintained at 2–3 times the measured convection requirement for oxygen in this species (Burggren and Cameron, 1980).

Heart rate (f_H) and dorsal aortic blood pressure (P_{DA}) were measured from the dorsal aortic cannulae using a pressure transducer (Micron MP-15D) connected to a two-channel thermal pen recorder (Dash II, Astromed) *via* a strain gauge preamplifier (Coulbourne). Ventilatory rate (f_G) and opercular pressure amplitude (P_{OP}) were measured from the opercular cannulae (PE-160) using a pressure transducer (Validyne DP45-34) and associated carrier demodulator (Validyne CD15). Opercular pressure amplitude was used to approximate changes in ventilatory effort.

Experimental procedure

After a stabilization period of approximately 30 min, when cardiovascular and ventilatory variables had reached a steady state, fish were exposed to aquatic hypoxia (P_{O_2}=6.1±0.5 kPa=46±4 mmHg) for 10 min by switching the submersible pump to another reservoir (also with 0.004 % MS-222) that had been

bubbled with N_2 to the desired P_{O_2}. Fish were returned to normoxia and allowed to recover for approximately 45 min. If cardiovascular and ventilatory variables were stable and near pre-hypoxia levels, the fish were then subjected to external NaCN administration by injecting into the ventilatory water flow either a water control or 500 μg of NaCN dissolved in 1 ml of dechlorinated tap water. The water bearing the NaCN was drained into a separate container to prevent recirculation of the NaCN past the gills. The effects of internal NaCN (50 μg in 0.25 ml of Cortland saline), given as a bolus injection into the DA, were further assessed in two fish from the completely denervated group ($IX+Xb_{1-4}$) to ensure that all reflexes were abolished. The NaCN and hypoxic exposure experiments were repeated in five fish in which cardiac reflexes were blocked with atropine sulphate (0.15 mg kg^{-1} injected *via* the DA cannula), so that the magnitude of the direct effects of NaCN or hypoxaemia on heart rate and blood pressure could be determined separately from chemoreflex responses. The efficacy of parasympathetic blockade was confirmed by giving acetylcholine (500 μg), *via* the DA cannulae, before proceeding.

Data analysis and statistics

Cardiovascular and ventilatory variables were analyzed in 30 s bins, for 1 min before and during 10 min of hypoxia, or for 15 min following NaCN administration. The effects of hypoxia and NaCN on cardiovascular and ventilatory variables were analyzed using a two-way analysis of variance (ANOVA) without replication (Sokal and Rohlf, 1981) to determine if there were significant changes over time. All statistics were performed on data before calculating the percentage change from normoxia (see Fig. 1) or pre-injection (see Fig. 3) for graphical display. Possible effects of total branchial denervation were examined by comparing resting, normoxic cardiovascular and ventilatory variables of the sham group and the nerve-sectioned groups using the T' method for multiple comparisons among means (Sokal and Rohlf, 1981).

Results

Responses to hypoxia

Sham-operated fish responded to hypoxia in the same manner as intact ASB fish in previous experiments (Burleson and Smatresk, 1989), i.e. with bradycardia, increased P_{DA} and increased ventilation. Resting, normoxic cardiovascular and ventilatory variables of the sham-operated group and the nerve-sectioned groups were not significantly different (Table 1).

Heart rate

All experimental groups of fish showed modest, albeit significant, decreases in f_H during hypoxia (Fig. 1A). The presence of bradycardia even in atropinized animals, however, suggested that the reduced heart rate during hypoxic exposure

Table 1. *Mean values (±S.E.) of cardiovascular and ventilatory variables in sham-operated, nerve-sectioned and atropinized catfish during normoxia and after 10 min of hypoxia*

Group	*N*	f_G (beats min^{-1})	P_{OP} (Pa)	f_H (beats min^{-1})	P_{DA} (Pa)
Normoxia*					
Sham	7	69.6±5.77	2.94±0.59	96.4±2.62	228±9.51
IX	8	52.9±10.9	4.80±0.69	96.6±3.04	197±31.2
IX+Xb_1	6	50.3±11.9	5.88±0.78	85.0±5.17	199±40.0
IX+Xb_{1-3}	5	61.4±11.2	2.06±0.59	106±6.33	264±44.2
IX+Xb_{1-4}	7	65.7±9.84	1.86±0.39	92.3±6.61	242±44.3
Atropine	5	57.2±9.54	2.16±0.49	99.2±2.69	229±23.3
Hypoxia (10 min)					
Sham	7	92.3±3.22	5.68±0.49	83.1±2.76	253±13.3
IX	8	73.1±12.1	8.62±0.88	82.4±2.47	206±33.5
IX+Xb_1	6	79.7±12.9	10.1±1.08	76.3±2.05	214±42.3
IX+Xb_{1-3}	5	78.0±13.2	4.70±0.98	89.2±2.29	245±46.2
IX+Xb_{1-4}	7	62.0±13.4	2.06±0.98	82.3±2.14	250±42.4
Atropine	5	86.8±1.93	6.76±1.37	85.6±1.19	221±26.1

*Resting, normoxic cardiovascular and ventilatory variables of sham-operated and IX+Xb_{1-4}-sectioned fish were not significantly different.

1 kPa=0.133 mmHg.

f_G, ventilatory rate; f_H, heart rate; P_{OP}, opercular pressure; P_{DA}, dorsal aortic pressure.

was not exclusively a result of reflex increases in inhibitory cholinergic activity to the heart.

Blood pressure

Branchial nerve section significantly altered the pressor response to hypoxia (Fig. 1B). Sham-operated, IX denervates and atropinized animals showed significant changes in P_{DA} during 10 min of hypoxia. The response to hypoxia in these groups was characterized by an initial increase followed by a decrease in P_{DA}. Dorsal aortic blood pressure did not change significantly during hypoxia after section of IX+Xb_1, IX+Xb_{1-3} or IX+Xb_{1-4} (Fig. 1B). Atropinized animals showed a significant pressor response to hypoxia.

Ventilation

Total branchial denervation abolished the increased gill ventilation rate elicited by hypoxia. Gill ventilation rate increased significantly during 10 min of aquatic hypoxia in all treatment groups during hypoxia, except in completely denervated fish (Fig. 1C). Partial denervation had little effect on the response of f_G to hypoxia. If, during the nerve section operation, one of the branchial nerves was left intact (unilaterally), ventilation still increased during hypoxia but the response was

atropinized animals and partial branchial denervates by external NaCN (Fig. 3C). The response became progressively attenuated as more branchial nerves were sectioned, but was only abolished by complete denervation (Fig. 3C).

Changes in P_{OP} in response to NaCN (Fig. 3D) were similar to the f_G responses.

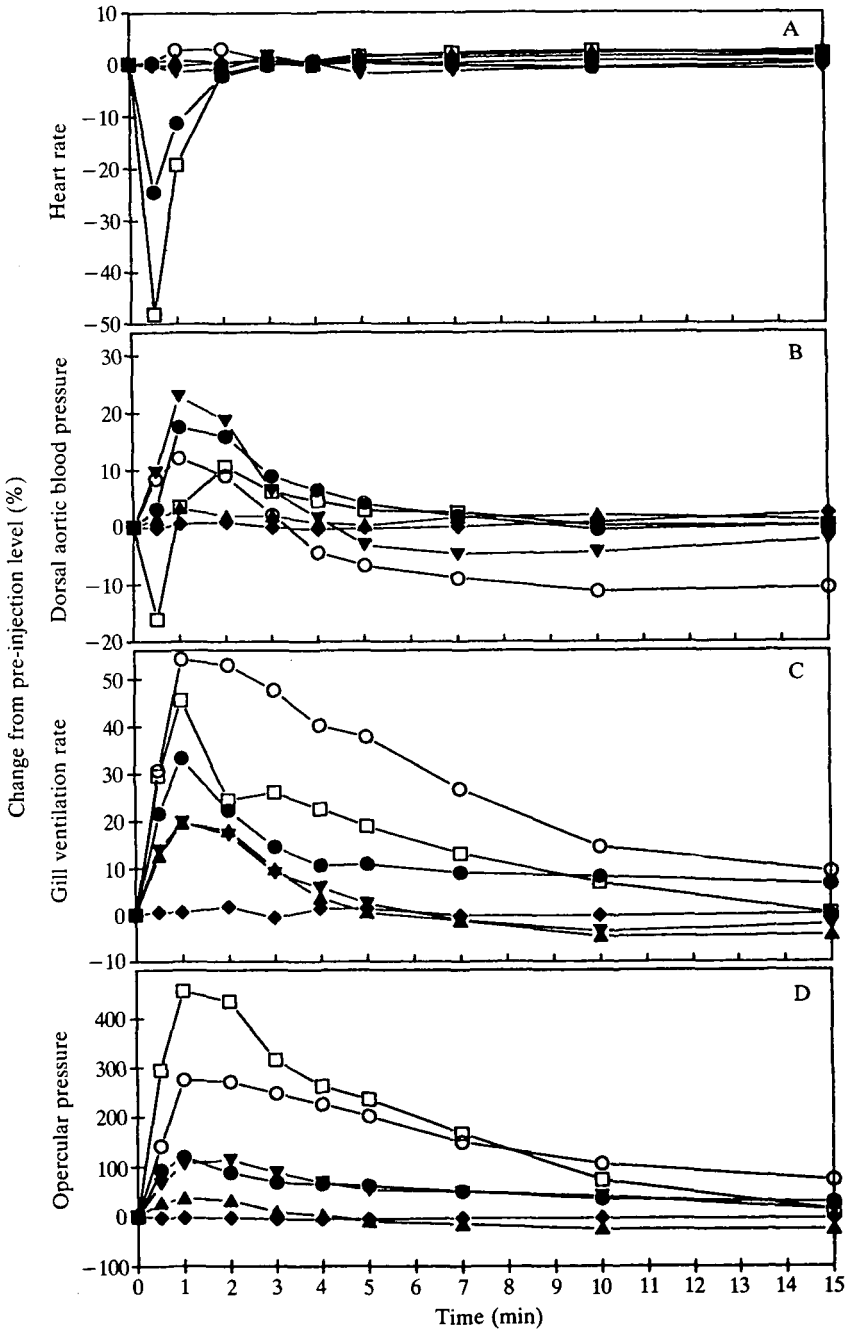

Fig. 3

Fig. 1. Cardiovascular and ventilatory responses of sham-operated (□), IX-sectioned (■), IX+Xb_1-sectioned (●), IX+Xb_{1-3}-sectioned (▲), IX+Xb_{1-4}-sectioned (◆) and atropinized (○) catfish during 10 min of aquatic hypoxia. (A) Heart rate (f_H) showed a significant decrease over time in all groups. (B) Dorsal aortic blood pressure (P_{DA}) changed significantly over time in sham-operated, IX-sectioned and atropinized groups. (C) Gill ventilation frequency (f_G) changed significantly over time in all groups except in the IX+Xb_{1-4}-sectioned group. (D) Opercular pressure amplitude (P_{OP}) changed significantly over time in all groups except in the IX+Xb_{1-4}-sectioned group. Values are mean percentage difference from normoxic values.

attenuated. Atropinization had no significant effect on the response of f_G to hypoxia.

Effects of nerve sections on the responses of P_{OP} to hypoxia were similar to the effects on f_G. P_{OP} was significantly stimulated in all experimental groups except in completely denervated animals (Fig. 1D).

Responses to NaCN

NaCN given externally into the respiratory water flow caused a transient bradycardia, hyperventilation and elicited a biphasic P_{DA} response in sham-operated animals (Figs 2 and 3). Fig. 2 is a representative trace showing the responses of a sham-operated fish to NaCN and the responses of the same fish after total branchial denervation.

Heart rate

Bradycardia in response to NaCN was somewhat attenuated, but still significant, after denervation of the first gill arch (nerves IX+Xb_1) (Fig. 3A). Section of the three posterior branchial vagi (nerves Xb_{1-3}), leaving IX and Xb_4 intact, abolished the transient bradycardia in response to external NaCN (Fig. 3A). Section of IX+Xb_{1-3}, IX+Xb_{1-4} and atropine also resulted in an abolition of the response of f_H to external NaCN.

Blood pressure

Sham-operated animals exhibited a biphasic response to external NaCN marked by an initial decrease then an increase in P_{DA} (Fig. 3B). The initial decrease in P_{DA} in the sham-operated fish was due to the severe bradycardia occurring at that time (Fig. 3A). When bradycardia was attenuated by nerve section or blocked by atropine there was no transient decrease in P_{DA}. After section of IX+Xb_1 or Xb_{1-3} the initial decrease in P_{DA} was abolished and P_{DA} showed a significant transient increase. The pressor response to external NaCN was abolished in fish where the glossopharyngeal and three or more of the branchial branches of the vagus were sectioned.

Ventilation

Gill ventilation frequency (f_G) was significantly stimulated in sham-operated,

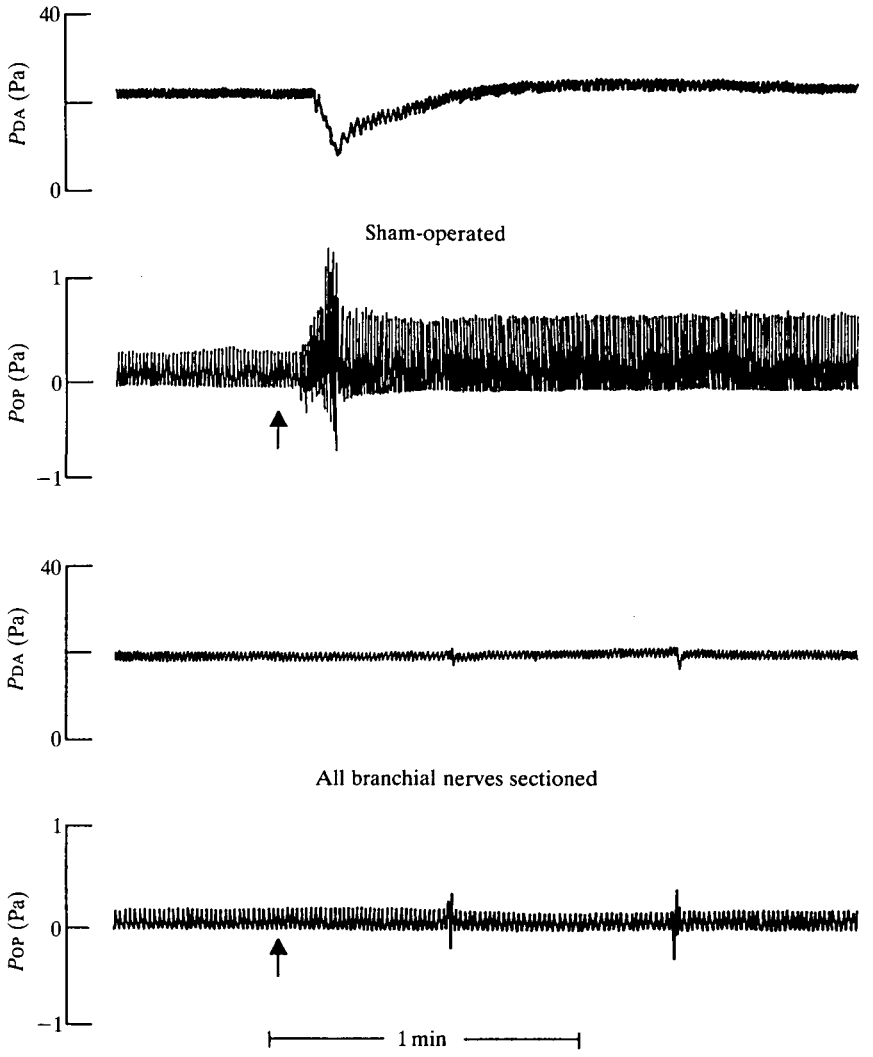

Fig. 2. Representative trace showing responses to 500 μg of NaCN given externally (at arrow) into the ventilatory water flow of a sham-operated fish (top two traces) and the same fish after total branchial denervation (bottom two traces). Upper trace in each pair shows P_{DA} and f_H. Bottom trace in each pair shows P_{OP} and f_G.

Fig. 3. Cardiovascular and ventilatory responses of sham-operated (□), IX+Xb_1-sectioned (●), Xb_{1-3}-sectioned (▼), IX+Xb_{1-3}-sectioned (▲), IX+Xb_{1-4}-sectioned (◆) and atropinized (○) catfish to 500 μg of external NaCN given at time zero. (A) Heart rate (f_H) showed a significant decrease in sham-operated and IX+Xb_1-sectioned groups. (B) Dorsal aortic blood pressure (P_{DA}) showed significant changes over time in all groups except the IX+Xb_{1-3} and IX+Xb_{1-4}-sectioned groups. (C) Gill ventilation frequency (f_G) showed a significant change over time in all groups except the IX+Xb_{1-4}-sectioned group. (D) Opercular pressure amplitude (P_{OP}) showed a significant change over time in all groups except the IX+Xb_{1-4}-sectioned group. Values are mean percentage difference from pre-injection values.

atropinized animals and partial branchial denervates by external NaCN (Fig. 3C). The response became progressively attenuated as more branchial nerves were sectioned, but was only abolished by complete denervation (Fig. 3C).

Changes in P_{OP} in response to NaCN (Fig. 3D) were similar to the f_G responses.

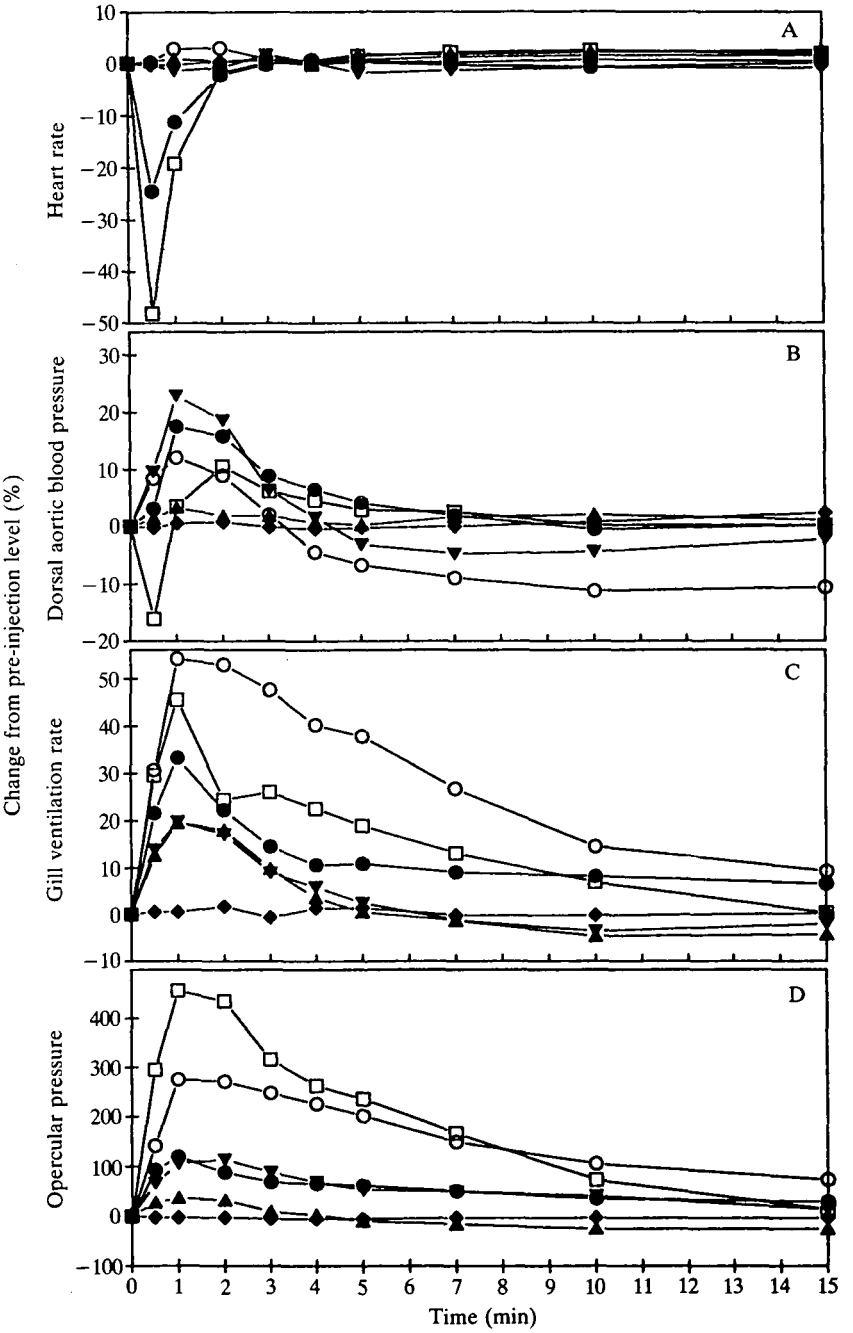

Fig. 3

Section of IX+Xb_1 or Xb_{1-3} severely attenuated the P_{OP} response. There was still a slight stimulation after section of IX+Xb_{1-3} but only total branchial denervation abolished the response of P_{OP} to external NaCN.

Internal NaCN (50 μg) was injected *via* the DA twice in each of two fish after complete branchial denervation. There were no cardiovascular or ventilatory responses to internal NaCN in these animals.

Discussion

Few experiments designed to delimit O_2-chemosensitive areas in fish have been conclusive. Three general locations were suggested by Hughes and Shelton (1962) as possible chemoreflexive regions: (1) the buccal, pharyngeal, branchial region in contact with the respiratory water flow, (2) the vasculature, either arterial or venous, and (3) within the tissues at the site of metabolism or in the brain.

It has become increasingly clear that fish possess O_2-sensitive chemoreceptors that monitor both internal (tissue or O_2 delivery) and external (water) O_2 levels. Hypoxic bradycardia is mediated exclusively by exteroreceptors in the sea raven (Saunders and Sutterlin, 1971), rainbow trout *Oncorhynchus mykiss* (Walbaum) [formerly *Salmo gairdneri* (Richardson)] (Smith and Jones, 1978; Daxboeck and Holeton, 1978) and coho salmon *Oncorhynchus kisutch* (Smith and Davie, 1984). In catfish, hypoxic reflex bradycardia is also mediated by external chemoreceptors, but ventilation is sensitive to both internal and external O_2 stimulus levels (Burleson and Smatresk, 1990). Milsom and Brill (1986) recorded O_2-sensitive afferent activity from the first gill arch of yellowfin tuna. They report that, of all the receptors responding to changing internal O_2 tensions, about half were also sensitive to external O_2 levels.

Cardiac responses to environmental hypoxia are attenuated somewhat by the vagolytic effect of MS-222 (Randall, 1962) in anaesthetized catfish (Burleson and Smatresk, 1989). However, catfish (Burleson and Smatresk, 1989) and other fish anaesthetized with MS-222 (Bamford, 1974; Smatresk *et al.* 1986) are still able to respond to hypoxia. The presence of bradycardia during aquatic hypoxia in all the experimental groups, including atropinized animals, suggests, however, that a large component of the observed bradycardia during this series of experiments was due to the direct depressant effect of hypoxia on the myocardium (Satchell, 1971) or perhaps a decrease in sympathetic tone (see Laurent *et al.* 1983). A baroreceptor-mediated bradycardia is unlikely, given that the lowest heart rate is concurrent with the lowest P_{DA} (Fig. 3A, B). Nevertheless, bradycardia in response to external NaCN is a vagal reflex and is abolished by atropine (Fig. 3A).

The cardiac responses to environmental hypoxia are mediated through cranial nerves IX and X to the first gill arch in salmonids (Daxboeck and Holeton, 1978; Smith and Jones, 1978; Smith and Davie, 1984) and cod (Fritsche and Nilsson, 1989). However, section of cranial nerves IX and X to the first gill arch in channel catfish attenuated but did not abolish bradycardia in response to NaCN (Fig. 3A). When nerves Xb_{1-3} are sectioned, leaving nerve IX intact, bradycardia in response

to NaCN is abolished, suggesting that nerve IX is not an essential pathway for chemosensory information controlling heart rate or that the vagolytic effect of MS-222 was enough to mask any increased vagal tone mediated *via* nerve IX. For example, Butler *et al.* (1977) suggest that vagal tone is a function of afferent input and that, after a portion of this input is removed, remaining afferents may not be adequate to elicit a response. It is difficult to determine how much each cranial nerve contributes to bradycardia; however, these data imply that the chemoreceptors mediating bradycardia in channel catfish do not reside solely in the first gill arch.

Previous denervation studies have focused on ventilatory and cardiac effects; therefore, little is known about the consequences of nerve section on blood pressure in fish. Saunders and Sutterlin (1971) report that pressor responses to hypoxia in the sea raven were little affected by bilateral section of either nerve IX or both nerves IX and X. In contrast, nerve sections have significant effects on pressor responses in channel catfish (Figs 1B, 3B). The increases in P_{DA} in response to NaCN after section of nerves IX+Xb_1 and Xb_{1-3} are similar (Fig. 3B); however, when nerves IX+Xb_{1-3} are sectioned the pressor response is greatly attenuated. It is not possible to separate the effects of baroreceptor, chemoreceptor and efferent motor neurone interference or local vascular reflexes on P_{DA} in this study; however, these results suggest that the gills contain all the sensory components needed to mediate pressor responses to hypoxia and NaCN.

Previous studies in which nerves IX and X have been bilaterally sectioned in fish have also found that there was little or no effect on resting, normoxic ventilatory patterns (Shelton, 1959; Hughes and Shelton, 1962; Saunders and Sutterlin, 1971). Deafferentation of the pseudobranch has no effect on the ventilatory response to hypoxia in rainbow trout (Randall and Jones, 1973) and, when the branches of nerves IX and X to the first gill arch are sectioned bilaterally, hypoxic bradycardia is abolished but ventilatory responses remain unaltered (Smith and Jones, 1978).

In contrast to previous nerve-section studies on tench (Hughes and Shelton, 1962) and sea raven (Saunders and Sutterlin, 1971), bilateral section of nerve IX and branchial branches of nerve X in channel catfish totally abolished the ventilatory responses to hypoxia (Figs 1 and 3). The persistence of hypoxic reflexes after cutting nerves IX and X indicates that, in the tench and sea raven, there are O_2-sensitive chemoreceptors innervated by nerves other than IX and X. The branchial nerves in fish are VII, IX and X (Nilsson, 1984); however, since channel catfish lack a pseudobranch (Grizzle and Rogers, 1976), IX and X are the branchial nerves in this species.

The pseudobranch may contain O_2-sensitive elements (Laurent and Rouzeau, 1972). Although surgical removal of the pseudobranch (Bamford, 1974) or deafferentation of the pseudobranch (Randall and Jones, 1973) has no effect on hypoxic ventilatory reflexes, it is conceivable that O_2-sensitive receptors in the pseudobranch could still stimulate ventilation in other species *via* cranial nerve VII during hypoxia after the gills have been denervated. A variety of partial branchial denervations never completely abolished ventilatory reflexes in catfish, indicating

that there is considerable redundancy of chemosensory innervation and that relatively little sensory input is necessary to elicit ventilatory hypoxic reflexes. This observation supports earlier work suggesting that teleosts possess a very diffuse O_2-chemoreceptive system (see Jones and Milsom, 1982, for a review).

Despite the suggestion that there are central O_2-sensitive chemoreceptors (Saunders and Sutterlin, 1971; Bamford, 1974; Jones, 1983) there is little experimental support for this view and it appears to have been developed as a default location due to the inability of previous studies to localize these receptors. Catfish have no significant ventilatory response to either hypoxia or NaCN after total branchial denervation. Hypoxia undoubtedly stimulated both external and internal receptors. While external NaCN predominantly stimulated external receptors, internal NaCN injections in two branchially denervated fish did not result in ventilatory stimulation. These data, along with the responses to hypoxia, suggest that internally oriented O_2 chemoreceptors were deafferented.

Central hypoxia has been shown to stimulate ventilation in some mammals, particularly during the foetal stage (Chernick, 1981). If other mechanisms do exist in fish, they probably have only negligible effects, since f_G actually begins to decrease after 10 min of hypoxia following branchial denervation (Fig. 1).

Conclusions about cardiovascular and ventilatory control based on nerve section data must be tempered by the fact that branchial innervation carries sensory information, in addition to O_2 chemoreception, which affects respiration. Mechanoreceptors, baroreceptors, nociceptors and chemoreceptors, other than O_2-sensitive ones, can alter cardiovascular and ventilatory variables (see Jones and Milsom, 1982, for a review). Branchial mechanoreceptors have been identified in elasmobranchs and teleosts and are sensitive to arch, raker and/or filament displacement (de Graaf *et al.* 1987; de Graaf and Ballintijn, 1987). The role of these receptors during normal ventilation is uncertain; however, possible roles include coupling of heart rate and ventilation (Satchell, 1960), and maintenance of the gill curtain during ventilation and gill arch position during feeding (de Graaf and Ballintijn, 1987).

Baroreceptors are present in all the gill arches and mediate a bradycardia in response to increased blood pressure (see Nilsson, 1984, for a review). Baroreceptors may also participate in the regulation of blood flow and lamellar recruitment *via* changes in branchial vascular resistance and, therefore, influence gas exchange and the internal oxygen stimulus levels. Nociceptor stimulation elicits apnoea, bradycardia and hypotension in response to harmful mechanical and chemical stimuli that may damage the delicate respiratory epithelium (Satchell, 1978).

Also in the gills of fish there exist receptors sensitive to a variety of chemical stimuli; however, the specificities and reflex effects of these receptors are not completely understood. Laurent and Rouzeau (1972) recorded increases in neural activity from the branch of nerve IX innervating the pseudobranch of trout in response to changes in pH, osmotic pressure and $[Na^+]$. Receptors showing high sensitivity to CO_2, a potential ventilatory stimulant, have been identified in the buccal cavities of carp (Konishi *et al.* 1969) and eel (Yoshii *et al.* 1979).

Nerve sections had no significant effect on resting, normoxic ventilatory patterns, indicating that none of the essential motor pathways necessary for ventilation were affected. However, the lack of a significant ventilatory response to either hypoxia or NaCN after total branchial denervation suggests that all hypoxic reflexes in channel catfish are mediated by peripheral O_2-sensitive chemoreceptors in the gills and that the sensory information from these receptors travels in the branchial branches of nerves IX and X.

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