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THE EFFECT OF SECTIONING CRANIAL NERVES V, VII, IX AND X ON THE CARDIAC RESPONSE OF THE DOGFISH *SCYLIORHINUS CANICULA* TO ENVIRONMENTAL HYPOXIA

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SUMMARY

1. Exposure of the dogfish to rapidly induced hypoxia caused an initial, large reduction in heart rate to 32% of its initial normoxic value. When P_{O_2} of inspired water (P_{I,O_2}) reached its lowest level, heart rate increased to 65% of its normoxic value and this rate was maintained until P_{I,O_2} was returned to normal.

2. Bilateral section of the branchial and pharyngeal branches of *either* cranial nerve IX *or* cranial nerve X had no significant effect on the cardiac response to rapidly induced hypoxia. Bilateral section of the pharyngeal and branchial branches of *both* of these cranial nerves abolished the initial large reduction in heart rate in response to rapidly induced hypoxia; heart rate fell steadily to 40% of its initial normoxic value after 3 min.

3. Bilateral section of cranial nerves V and VII had a similar effect, with heart rate reaching 62% of its normoxic value, 3 min after exposure to rapidly induced hypoxia. When the four cranial nerves were bilaterally sectioned together, there was no significant change in heart rate after 3 min exposure to rapidly induced hypoxia.

4. It is concluded that the oxygen receptors responsible for the bradycardia elicited in dogfish by environmental hypoxia are innervated by cranial nerves, V, VII, IX and X and that if receptors in the C.N.S. are involved in the response, their role is minimal.

INTRODUCTION

A reduction in environmental oxygen tension initiates a co-ordinated response by the respiratory and cardiovascular systems in all of the fish that have been studied so far. In teleosts, ventilation volume tends to increase, respiratory frequency and tidal volume both rise, heart rate is reduced and there is often a rise in cardiac stroke volume of equal magnitude (Randall & Shelton, 1963; Holeton & Randall, 1967*a*, *b*; Marvin & Heath, 1968; Eddy, 1974). In elasmobranchs, respiratory frequency and ventilation volume appear to change relatively little in response to environmental hypoxia, although there is a reduction in heart rate and an accompanying increase in cardiac stroke volume (Ogden, 1945; Hughes & Umezawa, 1968; Piiper, Baumgarten & Meyer, 1970; Butler & Taylor, 1971, 1975). The innervation of the receptors

responsible for the increase in ventilation volume and/or the bradycardia in teleosts and for the bradycardia in elasmobranchs, has not yet been fully determined.

Chemoreceptors, which can cause hyperpnoea or bradycardia when stimulated with hypoxic and/or hypercapnic blood, are present in Amphibia (Smyth, 1939), birds (Jones & Purves, 1970*a*, *b*) and mammals (Angell James & Daly, 1972). They are innervated by branches of the IX or X cranial nerves and are located close to the carotid and aortic arches. Similar receptors, therefore, may be associated with the branchial arches in fish and may be innervated by cranial nerves IX and/or X. However, neither the increase in ventilation volume in teleosts exposed to hypoxia (Hughes & Shelton, 1962) nor the bradycardia in elasmobranchs exposed to anoxia (Satchell, 1961) were abolished by bilaterally sectioning cranial nerves IX and X. In each case the responses were modified and Hughes & Shelton concluded that if other peripheral receptors exist in teleosts, they must be innervated by one or both of cranial nerves V and VII, whereas Satchell presented evidence to suggest that centrally placed receptors are present in elasmobranchs.

The object of the present investigation was to determine the effect of bilaterally sectioning cranial nerves V, VII, IX and X, either separately or in various combinations, on the cardiac response of the dogfish to hypoxia which was induced rapidly, i.e. within 1 min, and which was maintained for at least 3 min.

MATERIALS AND METHODS

Fifty-six dogfish (*Scyliorhinus canicula*) of either sex were used. Their body mass ranged from 0·48 to 1·03 kg. Thirty of the experiments were carried out at the Marine Biological Association of the U.K., Plymouth, and, the remainder were performed on fish which were transferred from Plymouth to aquaria in Birmingham. In each case, the fish were acclimated to a temperature of 15 ± 1 °C for at least 1 week before the experiments and all experiments were performed at this temperature. The fish were anaesthetized in MS 222 and all surgical operations were performed in a room which was at the acclimation temperature. Cannulae were inserted into the dorsal and ventral aortae, caudal vein and orobranchial cavity (for details see Butler & Taylor, 1971, 1975).

In order to gain access to the IXth and Xth cranial nerves, the anterior cardinal sinus was displayed as described by Taylor, Short & Butler (1977) and then opened. While it was open, its posterior end was pinched closed between finger and thumb in order to reduce the amount of air entering the circulatory system. In 8 fish (shams) the branches of the IXth and Xth cranial nerves were displayed, but none of them was sectioned. Five fish had the IXth cranial nerve bilaterally sectioned, 6 fish had the branchial and pharyngeal branches of the vagus nerve bilaterally sectioned, and in 9 fish the IXth cranial nerve and the branchial and pharyngeal branches of the Xth nerve were bilaterally sectioned. In a further 5 fish the IXth and Xth pharyngeal and branchial branches were sectioned and the internal surface of the spiracle was destroyed by cautery, and in 2 of these animals a latex rubber mask was attached to the head in order to cover all of the sensory ampulla of that area. The points of nerve section can be seen in Fig. 1 and any reference to cutting cranial nerves IX and X means at the points shown. Note that the cardiac branches of the vagus w

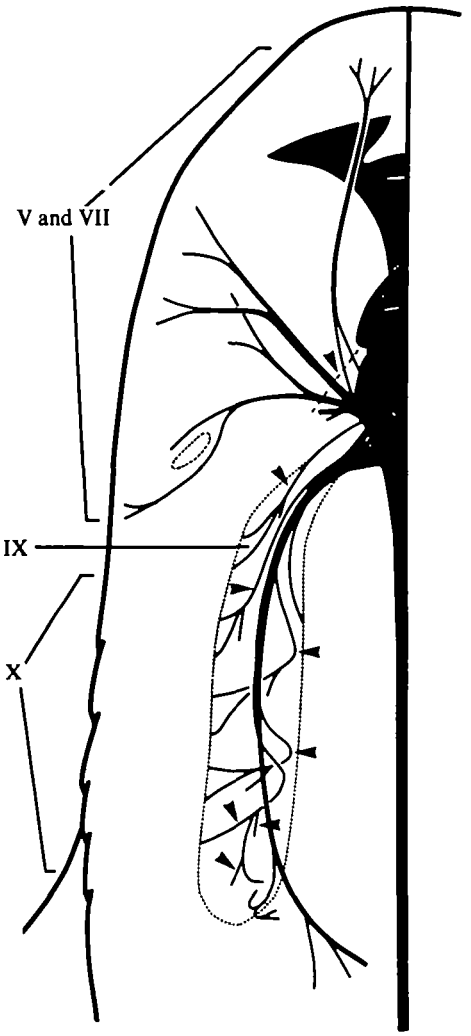

Fig. 1. Diagram of the left-hand side of the anterior region of the central nervous system of the dogfish *Scyliorhinus canicula* illustrating the position of cranial nerves V, VII, IX and X. The arrows indicate the points where these nerves were sectioned.

always left intact. Following the display or sectioning of the nerves, air was excluded from the circulatory system by laterally compressing the wall of the sinus and the muscle and skin were sutured in two layers. Any air remaining in the circulation was removed immediately, via the cannula in the ventral aorta. Blood loss during the entire operation was minimal and it was found from four animals that, compared with the value before the operation, there was no significant change in haematocrit 3 h after such an operation. The operation had a variable effect upon blood pressure (Table 1). Sometimes there was little effect (e.g. when the IXth and Xth nerves were sectioned) whereas on other occasions there was a significant reduction in ventral aortic pressure (e.g. when the Xth nerve was sectioned). These differences are probably fortuitous.

The Vth and VIIth cranial nerves were exposed and/or sectioned at their point of emergence from the medulla (Fig. 1). Two incisions were made in the chondro-

cranium, one either side of the mid-line. They extended from the region of the prosencephalon to the cerebellum and were joined anteriorly so that a flap of cartilage could be raised to expose the brain. In 4 fish (shams), the roots of cranial nerves V and VII were identified, but not sectioned; in 6 fish these nerves were bilaterally sectioned at their point of emergence from the medulla. Following identification or bilateral transection of the V and VII cranial nerves, the flap of chondrocranium was replaced to its original position and a piece of thick latex rubber was stuck over the region by Eastman 910 contact adhesive (Ciba-Geigy) in order to give a water-tight seal. Nine animals were completely deafferented, inasmuch as cranial nerves V and VII plus the pharyngeal and branchial branches of the IXth and Xth nerves were sectioned. The operation involved in exposing and sectioning cranial nerves V and VII had no significant effect on ventral aortic blood pressure (Table 1).

Bilaterally sectioning the branchial and pharyngeal branches of cranial nerves IX and X impaired ventilation by paralysing the parabranchial musculature, whereas bilaterally sectioning cranial nerves V and VII paralysed the orobranchial muscles. Nevertheless, the remaining respiratory muscles in each case were sufficient to maintain a reasonable, though somewhat reduced, P_{O_2} in the arterial blood (P_{a,O_2}) in these fish when in normoxic water. However, bilateral section of all four cranial nerves caused complete paralysis of the respiratory muscles; these fish were therefore artificially irrigated at a rate of 0·55 l min^{-1} via a tube placed in the orobranchial cavity.

Four fish were paralysed with pancuronium bromide (Pavulon: Organon Laboratories Ltd.) which was administered at a rate of 1·5 mg kg^{-1} h^{-1}. This dose stopped respiratory movements completely but did not have any vagolytic effect as indicated by electrical stimulation of the peripheral cut end of the branchial branch of the cardiac vagus. These fish were also artificially irrigated, at the same rate as the fish in which the respiratory muscles had been paralysed by nerve section.

The fish, in which the IXth and/or Xth cranial nerves were to be exposed and sectioned, were allowed to recover from the anaesthetic for at least 3 h after cannulation of the blood vessels and of the orobranchial cavity (i.e. before the anterior cardinal sinuses had been exposed). They were then subjected to a rapid reduction in inspired P_{O_2} (P_{I,O_2}) which fell from approximately 150 mmHg to 30 mmHg within 1 min (for details see Butler & Taylor, 1971). Oxygen tension in the inspired water and in the arterial blood were measured before and 2 min after the induction of hypoxia. Hypoxia was maintained for a total of 3 min, after which the fish were returned to normoxia. After this initial exposure to rapid hypoxia, the fish were re-anaesthetized and the required branches of the IXth and/or Xth cranial nerves were sectioned. Three hours after recovery from this, the fish were again subjected to rapid hypoxia. Thus the fish served as their own controls, with the effect of rapid hypoxia being studied before and after exposure and/or section of branches of the IXth and/or Xth cranial nerves. As the results from the control condition were always similar, it was not considered necessary to perform this elaborate procedure for those animals in which the Vth and VIIth cranial nerves were sectioned. These fish were merely exposed to rapid hypoxia after exposure and/or section of the nerves.

Any fish that showed a cardiac response to rapid hypoxia was injected with 0·2 mg kg^{-1} atropine sulphate (Sigma) and re-exposed to rapid hypoxia 10 min later.

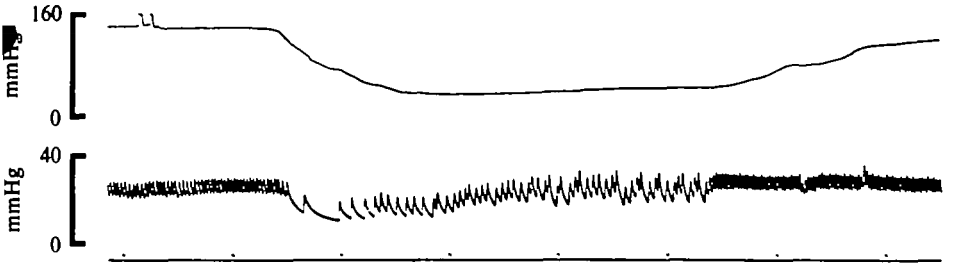

Fig. 2. Original trace of ventral aortic blood pressure from a dogfish (♂, 0·75 kg) to show the effect upon heart rate of rapidly induced hypoxia. Traces are, from above downwards: P_{I,O_2}, ventral aortic blood pressure, time marker (min).

Dorsal and ventral aortic blood pressures were measured by Bell and Howell 4-327-L221 transducers, orobranchial pressures by a S.E. Laboratories S.E. 4–86 transducer and P_{I,O_2} by a Radiometer oxygen electrode. The outputs from these were displayed on a 4-channel recorder (Devices Ltd). Oxygen tension in arterial blood was measured by a Radiometer electrode housed in a cuvette at 15 °C.

The means of the measured variables are expressed ± S.E. of mean. Student's *t* test was used to test the significance of any difference between two mean values. The word 'significant' in the present report means significant at the 95% confidence level ($P < 0.05$).

RESULTS

Controls and shams

The responses of the respiratory and cardiovascular systems of the control dogfish to rapidly induced hypoxia were similar to those reported by Butler & Taylor (1971) and are shown in Figs. 2 and 3(*a*). During the period when P_{I,O_2} fell rapidly there was a marked reduction in heart rate to an average of 32% of the initial normoxic value. This low heart rate was maintained for a few beats and is referred to as the transient bradycardia. Following the period when P_{I,O_2} was being reduced, i.e. when it had reached a steady value, heart rate increased to a mean level which was 65% of its initial normoxic value. This heart rate, which was maintained throughout the period of hypoxia, is called the stable bradycardia. After 3 min the water was re-aerated and heart rate increased to a value that was a few beats higher than the initial normoxic level. There was no significant change in respiratory frequency during hypoxia (Table 1). Following injection of atropine there was a significant, 32% increase in heart rate above the initial normoxic value and the cardiac response to subsequent rapid hypoxia was completely abolished. Similar responses to rapid hypoxia and to atropinization were obtained from the two groups of sham-operated fish (i.e. those fish in which the IXth and Xth cranial nerves were displayed and those in which the roots of cranial nerves V and VII were displayed) (Figs. 3*b*, 5*b*).

Cranial nerve sections

Bilateral sectioning of the branchial and pharyngeal branches of *either* the IX *or* X cranial nerves had no significant effect on the response of heart rate to rapidly induced hypoxia or to subsequent atropinization (Fig. 3*c*, *d*). There was, however, a dramatic change in the response to rapid hypoxia when the branchial and pharyngeal

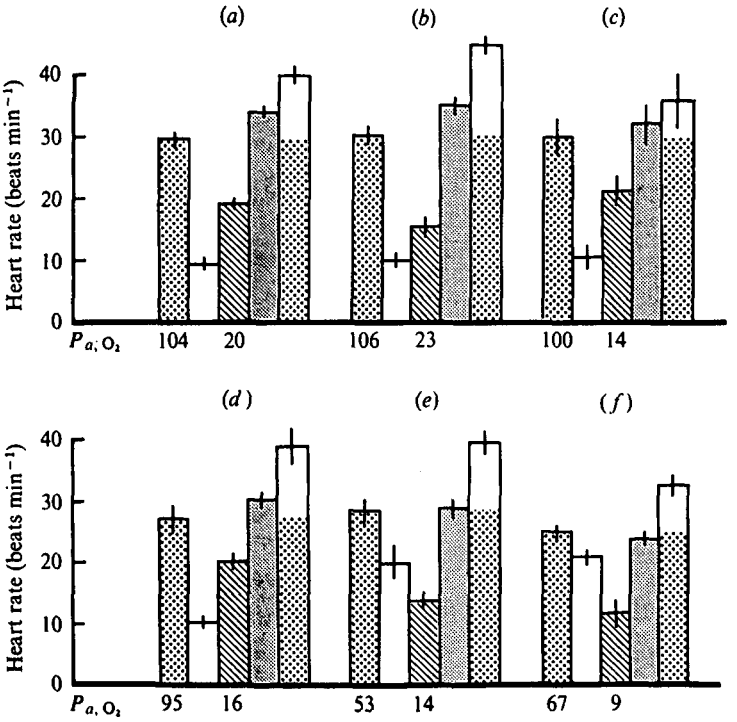

Fig. 3. The mean values (±S.E. of mean) of heart rate from dogfish exposed to rapidly induced hypoxia before and after exposure and/or sectioning cranial nerves IX or X. The various experimental categories are as follows: (*a*) control, (*b*) IX and X sham, (*c*) IX sectioned, (*d*) X sectioned, (*e*) IX and X sectioned, (*f*) IX and X sectioned, spiracle cauterized and the head covered with latex rubber. In each series, the histograms denote the initial normoxic heart rate (▦), the lowest heart rate recorded as P_{I,O_2} was falling (□) the heart rate 2 min after P_{I,O_2} was stable at approximately 30 mmHg (▨) the heart rate when P_{I,O_2} was returned to approximately 150 mmHg (▩). The final histogram shows the increase in heart rate (clear area) above the initial normoxic value (large dots) after injecting atropine (0·2 mg kg^{-1}). Mean values of P_{a,O_2} (mmHg) are given for normoxia and for the stable period during hypoxia for each experimental category.

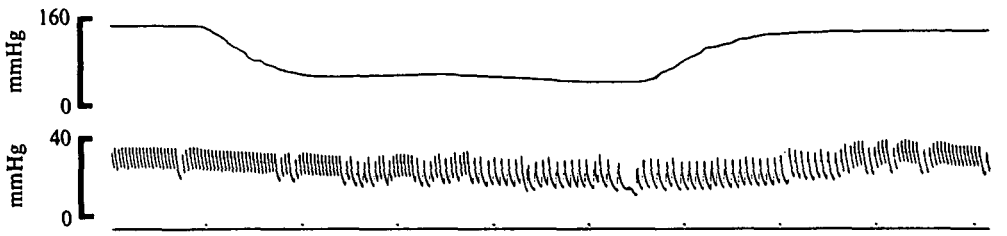

Fig. 4. Original trace of ventral aortic blood pressure from same dogfish as in Fig. 3 to show effect upon heart rate of rapidly induced hypoxia after bilateral sectioning of the pharyngeal and branchial branches of cranial nerves IX and X. Traces are, from above downwards: P_{I,O_2}, ventral aortic blood pressure, time marker (min).

branches of cranial nerves IX *and* X were bilaterally sectioned (Fig. 4). In this case there was no transient bradycardia; instead heart rate decreased steadily to, on average, 49% of its initial normoxic value during the 3 min period of hypoxia. Atropinization caused a 48% increase in the normoxic heart rate and abolished the progressive bradycardia in response to rapid hypoxia. After section of cranial nerves IX

Table 1. *Mean values* ± S.E. *of mean of respiratory frequency and mean blood pressure in the dorsal and ventral aortae before (grouped control) and after the various surgical procedures*

(Values are given during normoxia ($P_{I,O_2} \simeq 150$ mmHg) and during the stable phase of hypoxia following the rapid reduction of P_{I,O_2}. During this stable phase $P_{I,O_2} \simeq 30$ mmHg. The number of animals contributing to each mean value is given in parentheses.)

	Normoxia	Hypoxia
	Respiratory frequency (min^{-1})	
Grouped control	48·9 ± 1·4 (22)	52·6 ± 1·2 (22)
IX and X sham	54·8 ± 4·3 (4)	57·3 ± 4·4 (4)
IX cut	52·6 ± 2·3 (5)	52·8 ± 2·2 (5)
X cut	47·0 ± 3·7 (5)	47·2 ± 3·5 (5)
IX and X cut	45·3 ± 2·7 (3)	45·0 ± 2·1 (3)
V and VII sham	—	—
V and VII cut	49·9 (2)	46·5 (2)
V, VII, IX and X cut	—	—
	Mean blood pressure in the ventral aorta (mmHg)	
Grouped control	25·3 ± 1·1 (28)	23·1 ± 1·1 (28)
IX and X sham	20·5 ± 2·4 (6)	15·7 ± 1·9 (6)
IX cut	21·0 ± 1·7 (5)	22·0 ± 1·9 (5)
X cut	17·3 ± 0·9 (6)	17·2 ± 1·2 (6)
IX and X cut	24·8 ± 2·4 (9)	20·8 ± 2·7 (8)
V and VII sham	25·7 ± 2·2 (4)	23·8 ± 1·6 (4)
V and VII cut	25·1 ± 1·9 (6)	22·3 ± 3·1 (6)
V, VII, IX and X cut	26·9 ± 1·7 (9)	20·8 ± 1·4 (9)
	Mean blood pressure in the dorsal aorta (mmHg)	
Grouped control	23·1 ± 1·4 (15)	20·8 ± 1·5 (15)
IX and X sham	17·0 ± 2·8 (5)	13·0 ± 1·8 (5)
IX cut	—	—
X cut	15·5 (2)	13·5 (2)
IX and X cut	21·5 ± 0·9 (6)	14·8 ± 1·8 (6)
V and VII sham	—	—
V and VII cut	—	—
V, VII, IX and X cut	—	—

and X, P_{a,O_2} of fish in normoxic water was substantially lower than it was for fish in the control and sham-operated experimental categories (Fig. 3*e*). This is not surprising as the animals' irrigation system had been impaired by the nerve section. Therefore although P_{O_2} of the surrounding water was falling at the same rate as in the earlier experiments, the fish may not have been experiencing such a rapid reduction in P_{I,O_2} and/or P_{a,O_2} during hypoxia, because ventilation volume may not have been as high as in the control fish. This possibility was tested by paralysing fish with pancuronium bromide, artificially irrigating them and then exposing them to rapid hypoxia before and after sectioning cranial nerves IX and X. The response of the heart was not significantly different in the paralysed fish from that described for spontaneously breathing fish. Thus, the cardiac response to rapid hypoxia after section of cranial nerves IX and X cannot be explained in terms of impaired water flow or gas exchange. Neither is it, to any obvious extent, the result of stimulating receptors associated with the pseudobranch or with the skin in the head region, because

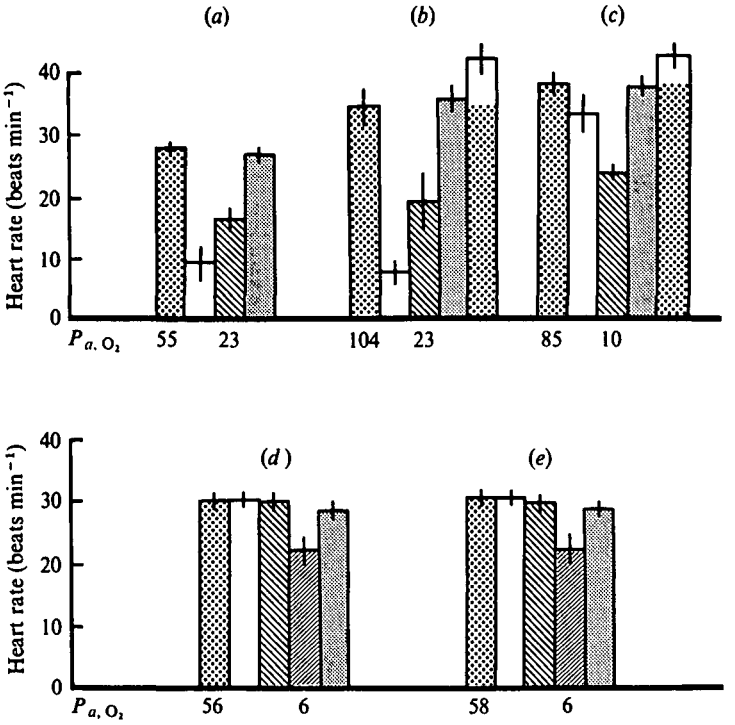

Fig. 5. Mean values (±S.E. of mean) of heart rate from dogfish exposed to rapidly induced hypoxia after injection of pancuronium bromide, exposure and/or section of cranial nerves V and VII, or section of cranial nerves, V, VII, IX and X. The various experimental categories are as follows: (*a*) injection of pancuronium bromide (1·5 mg kg^{-1} h^{-1}), (*b*) V and VII sham, (*c*) V and VII sectioned, (*d*) V, VII, IX and X sectioned, (*e*) V, VII, IX and X sectioned + atropine (0·2 mg kg^{-1}). In each series, the histograms denote the initial normoxic heart rate (▦), the lowest heart rate recorded as P_{I,O_2} was falling (□), the heart rate 2 min after P_{I,O_2} was stable at approximately 30 mmHg (▧), the heart rate when P_{I,O_2} was returned to approximately 150 mmHg (▩). In (*b*) and (*c*) the final histogram shows the increase in heart rate (clear area) above the initial normoxic value (large dots) after injecting atropine (0·2 mg kg^{-1}). In (*d*) and (*e*) the heart rate is given after 10 min exposure to hypoxia (▨). Mean values for P_{a,O_2} (mmHg) are given for normoxia and for the stable period during hypoxia for each experimental category. For (*d*) and (*e*) the hypoxic P_{a,O_2} was taken 10 min after exposure to hypoxia.

cauterizing the spiracles and/or covering the outside of the head with latex rubber, in addition to bilaterally sectioning cranial nerves IX and X, did not abolish or modify the slowly developing bradycardia which occurred during rapid hypoxia (Fig. 3*f*). In fish paralysed with Pavulon, perfused with normoxic water and with all cranial nerves intact, P_{a,O_2} was 55 ± 14 mmHg. This is similar to the value recorded in spontaneously breathing fish in normoxic water after bilaterally sectioning cranial nerves IX and X (Figs. 3*e*, 5*a*).

Sectioning cranial nerves V and VII also abolished the transient bradycardia during rapid hypoxia (Fig. 5*c*) and left a slowly developing reduction in heart rate which could be abolished by injection of atropine. After 3 min exposure to hypoxia in non-atropinized fish, heart rate was 62% of its initial hypoxic level. Thus, the reduction in heart rate was somewhat less in these fish compared with those in which cranial nerves IX and X were bilaterally sectioned, although P_{a,O_2} reached during hypoxia

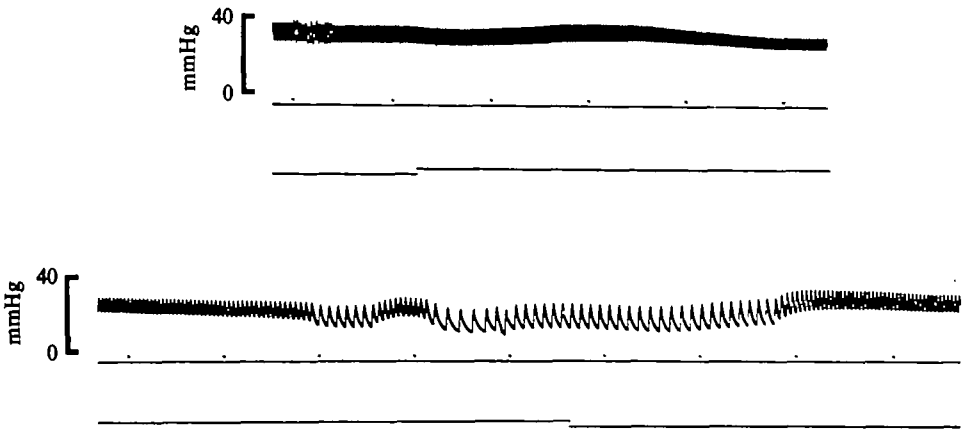

Fig. 6. Original trace of ventral aortic blood pressure from a dogfish (♀ 0·53 kg) to show the effect upon heart rate of rapidly induced hypoxia after bilateral sectioning of cranial nerves V, VII, IX and X. The traces, are from above downwards: Ventral aortic blood pressure, time marker (min) event marker (up indicates point where P_{I,O_2} begins to fall, down indicates point where P_{I,O_2} begins to rise). The traces are discontinuous; the first deflexion on the time marker in the second section is 10 min after the onset of hypoxia.

similar in each case (Figs. 3*e*, 5*c*). Other differences between the two groups of animals were that injection of atropine gave rise to a relatively small (10%) increase in heart rate in fish after bilateral sectioning of cranial nerves V and VII and that P_{a,O_2} fell less in fish in normoxic water after sectioning nerves V and VII than after cutting nerves IX and X (Figs. 3*e*, 5*c*). The effect of sectioning cranial nerves V and VII upon the cardiac response to rapid hypoxia was similar in artificially irrigated fish paralysed with Pavulon and in spontaneously breathing fish.

As with the control fish, there was no change in respiratory frequency during hypoxia following any combination of nerve sectioning mentioned so far; neither did any of these nerve lesions themselves have a significant effect on respiratory frequency (Table 1).

The final group of experimental fish was those in which cranial nerves V and VII plus the pharyngeal and branchial branches of nerves IX and X were sectioned. This led to a complete cessation of breathing movements so that these animals had to be artificially irrigated. It has been shown in the experiments with fish paralysed by Pavulon, that the cardiac response to rapid hypoxia in animals with an intact nervous system was similar in both artificially irrigated and spontaneously breathing fish. Thus, the complete absence of any change in heart rate after 3 min exposure to rapid hypoxia in the last group of experimental fish (see Fig. 5*d*) can perhaps be attributed to the complete removal of all of the afferent pathways which are involved in the response seen in the control animals. These fish (i.e. those whose cranial nerves V, VII, IX and X had been sectioned) were in fact exposed to hypoxia for at least 10 min by which time there was a significant reduction in heart rate to 73% of its normoxic level (Fig. 6), but this was not abolished by injection of atropine (Fig. 5*e*) and was thus, possibly, the direct effect of hypoxia upon the myocardium. Another unique feature about this group of fish was that injection of atropine caused no significant increase in heart rate.

DISCUSSION

The operative procedures used in the present investigation did not themselves have any major effect upon the physiological state of the fish; thus any significant change associated with nerve sectioning can be justifiably attributed to the removal of the influence of the nerves in question. The experiments with Pavulon indicate that any modification of the cardiac response to hypoxia by nerve section results from the removal of afferent and not of efferent activity. The reduction in P_{a,O_2} associated with sectioning the branchial and pharyngeal branches of cranial nerves IX and X or with sectioning cranial nerves V and VII could have resulted from impaired water flow. However, fish paralysed with Pavulon and artificially irrigated had a similar P_{a,O_2} in normoxic water as those fish in which nerves IX and X had been sectioned, and this was less than in those fish with nerves V and VII sectioned. The fall in P_{a,O_2} was apparently related directly to the removal of efferent activity, affecting the dynamics and regional distribution of water flow, and *not* to any resultant reduction in total water flow. The implication is, therefore, that the orientation of the gills themselves is adversely modified after paralysis. This may be as a result of the relaxation of intrinsic gill muscles (cf. Pasztor & Kleerekoper, 1962) and/or of muscles associated with the branchial arches (Hughes & Ballintijn, 1965). There was no evidence of any increase in respiratory frequency following bilateral section of pharyngeal and branchial branches of the IXth and Xth nerves. Surprisingly, therefore, it seems that the inhibitory reflex, described by Satchell (1959) in *Squalus acanthias*, is not present in *Scyliorhinus canicula*.

The absence of any change in heart rate after sectioning cranial nerves IX and X was also surprising. Irving, Solandt & Solandt (1935) demonstrated that baroreceptor reflexes are mediated via branchial branches of cranial nerves IX and X in the elasmobranch. Elimination of afferent activity from such receptors (physiologically similar to a reduction in blood pressure) might be expected to cause an increase in heart rate. There was, however, a tachycardia following section of cranial nerves V and VII and subsequent atropinization caused a smaller increase in heart rate than it did in the control animals. Thus, afferent input from the Vth and VIIth nerves contributes substantially to the generation of cardiac vagal tone. As additional transection of nerves IX and X removed all remaining vagal tone to the heart, these four cranial nerves appear to provide all of the sensory input that is necessary for the central generation of vagal activity to the heart of dogfish. These cranial nerves carry afferent fibres from sense organs other than the oxygen receptors. Salmoiraghi & Burns (1960) described how progressive isolation of the medulla in cats reduced the number of neurones with respiratory activity. It is possible, therefore, that the central generation of vagal tone to the heart in dogfish is dependent upon a general level of afferent input which is provided by the various types of receptors innervated by cranial nerves V, VII, IX and X. Absence of this general input may not only remove any cardiac vagal tone, it may also make it more difficult for any remaining afferent input, which would normally increase efferent vagal activity to the heart, to have its effect. This was suggested by the fact that pinching the skin, which in intact fish causes a substantial bradycardia, had a reduced effect in fish after sectioning cranial nerves V, VII, IX and X. The complete abolition of the bradycardia during hypoxia after the four cran

nerves had been bilaterally sectioned does not necessarily mean that all pathways from oxygen receptors had been interrupted; it could mean that sufficient general sensory input had been removed to render any afferent activity from remaining oxygen receptors insufficient to generate vagal activity to the heart. Thus, the present investigation does not wholly contradict those authors (Satchell, 1961; Saunders & Sutterlin, 1971; Bamford, 1974) who believe that receptors in the central nervous system are responsible for the bradycardia or hyperpnoea that remain after the pharyngeal and branchial branches of cranial nerves IX and X have been severed. Indeed, the removal of the immediate, intense bradycardia following section of the IXth and Xth nerves and the subsequent progressive bradycardia seen in dogfish exposed to rapid hypoxia, are similar to the results obtained by Satchell and would, by themselves, support his conclusion. On the other hand, our results vindicate those authors (Hughes & Shelton, 1962; de Kock, 1963; Saunders & Sutterlin, 1971) who have suggested that peripheral receptors innervated by cranial nerves V and VII could be involved in the response to hypoxia. This has now been clearly demonstrated to be so for the dogfish. It is also clear that the spiracle and outside skin of the head are not the predominant locations for these receptors. It is likely that they are distributed over the surface of the lips, mouth and pharynx. Although oxygen receptors may be widespread throughout the buccopharynx they may also be sparse, which could explain why it has not been possible to detect any change in activity in palatine, facial or branchial branches of these four cranial nerves when a fish is exposed to hypoxia (Konishi *et al.* 1969; Sutterlin & Saunders, 1969). The existence of receptors thinly spread over a wide area is also consistent with the results of the present experiments. The full response to rapidly induced hypoxia is modified when either the Vth and VIIth or the IXth and Xth nerves are bilaterally sectioned. Cutting *either* nerve IX *or* nerve X alone has no effect on the response.

It is not possible from the present experiments to determine whether the oxygen receptors respond to changes in P_{I,O_2} or P_{a,O_2}. However, since cranial nerves V and VII innervate the orobranchial cavity, the pharynx, the spiracle and the sensory ampullae in the snout, it is possible that the majority of the oxygen receptors innervated by these nerves detect changes in P_{O_2} of the water. Cranial nerves IX and X supply the pharynx and the gill arches, so again it is possible that superficially placed oxygen receptors would detect P_{O_2} of the water. These two cranial nerves also richly innervate the branchial blood vessels and their arterioles in both elasmobranchs (Boyd, 1936) and teleosts (de Kock, 1963). Oxygen receptors in these regions would be ideally situated to detect P_{O_2} of the blood.

It is proposed, therefore, that in the dogfish and possibly in teleosts, peripheral receptors are largely, if not completely responsible, for the hyperpnoea (in teleosts) and the bradycardia. They are located widely in the orobranchial (buccal) and parabranchial (opercular) cavities and are innervated by cranial nerves V, VII, IX and X. Also, the majority of these receptors must be stimulated in order to produce the complete response to hypoxia. The phylogenetic fate of these receptors may have been different. Those innervated by the IXth and Xth nerves most likely retained their sensitivities to hypoxia and developed into the discrete carotid and aortic bodies of the terrestrial vertebrates. Those innervated by the Vth and VIIth nerves may have completely disappeared and left those receptors that are sensitive to other chemicals

(Konishi & Hikada, 1969; Konishi *et al.* 1969) or they may have become sensitive to other chemicals themselves. In mammals there are receptors around the mouth and in the nasopharynx which are innervated by the Vth cranial nerve and which, when stimulated by water (Angell James & Daly, 1972) or noxious chemicals such as cigarette smoke (McRitchie & White, 1974), cause a reflex bradycardia. If receptors in the central nervous system are involved in the cardiac response of the dogfish to hypoxia, then they would appear to be of minimal importance.

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