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THE USE OF ALPHA-METHYL-*p*-TYROSINE TO CONTROL CIRCULATING CATECHOLAMINES IN THE DOGFISH *SCYLIORHINUS CANICULA*: THE EFFECTS ON GAS EXCHANGE IN NORMOXIA AND HYPOXIA

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Summary

We assessed the effectiveness of alpha-methyl-*p*-tyrosine in inhibiting catecholamine synthesis in the dogfish and examined the effects of catecholamine depletion on the cardiovascular system in normoxia and in response to hypoxia.

Although alpha-methyl-*p*-tyrosine (50 mg day^{-1} intraperitoneally for 5 days) substantially reduced the dogfish's ability to increase the level of circulating catecholamines in response to hypoxia, it also substantially reduced normoxic oxygen consumption in the whole animal, an observation not previously reported. Metabolic studies on isolated dogfish hepatocytes indicate that this is a direct effect on oxidative metabolism at the cellular level rather than any effect on the oxygen delivery function of the fish's cardiovascular system. Despite the effects of alpha-methyl tyrosine on normoxic oxygen consumption, the present results indicate that the lack of any large increase in the circulating levels of catecholamines in response to hypoxia in fish treated with alpha-methyl tyrosine does not compromise their gas exchange ability.

Introduction

The potential control of the cardiovascular system by circulating catecholamines is of particular interest in elasmobranchs since these fish lack any direct sympathetic innervation of the heart (Young, 1933; Gannon & Burnstock, 1969; Short *et al.* 1977) and possess no motor innervation of their gill blood vessels (Metcalfe & Butler, 1984*b*); consequently, it would seem that humoral control of the heart and branchial circulation is the only type of extrinsic control in these animals. Furthermore, when their environmental oxygen level is reduced, dogfish release comparatively large quantities of adrenaline and noradrenaline into their

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bloodstream (Butler *et al.* 1978), suggesting that these hormones may play some role in their cardiovascular response to hypoxia.

The physiological role of the increase in circulating catecholamines in elasmobranchs in response to hypoxia has been of interest in our laboratory for a number of years. However, manipulative experiments designed to elucidate their function have presented a number of problems. First, the diffuse anatomical arrangement of the chromaffin tissues in this species makes procedures such as surgical denervation or removal of the chromaffin tissues impracticable, whereas studies using adrenergic antagonists require close confinement of the fish to measure oxygen consumption in the relatively short period that these drugs are effective (Metcalfe & Butler, 1988). We therefore investigated the possibility of long-term inhibition of catecholamine synthesis to prevent increases in circulating catecholamine levels in response to hypoxia.

In mammals, catecholamines are synthesized from tyrosine *via* DOPA→dopamine→noradrenaline→adrenaline (Holtz, 1939; Blaschko, 1939), where the conversion of tyrosine to DOPA is the rate-limiting step (Nagatsu *et al.* 1964; Levitt *et al.* 1965). The enzyme which catalyses this reaction, tyrosine hydroxylase, can be inhibited by alpha-methyl-*p*-tyrosine (Spector *et al.* 1965) and can effectively reduce catecholamine synthesis in man (Sjoerdsma *et al.* 1965) and other mammals (Porter *et al.* 1965). In teleosts, a similar biosynthetic pathway for adrenaline and noradrenaline appears to exist (Jönsson & Nilsson, 1983). However, we know of no published reports of the effects of alpha-methyl tyrosine on catecholamine synthesis in lower vertebrates. Nonetheless, this drug seems an ideal tool with which to study the effects of catecholamine depletion on the response to hypoxia in the dogfish.

In this study we investigated the effectiveness of alpha-methyl tyrosine in inhibiting catecholamine synthesis in the dogfish, and the consequent effects of this inhibition on the cardiovascular system in normoxia and the subsequent response to hypoxia.

Materials and methods

Experiments on intact animals were performed on 25 dogfish of either sex, the mass of which ranged from 0·51 to 1·08 kg. The fish were obtained either from the MBA, Plymouth or from the Marine Biology Laboratory, UNCW, Menai Bridge, Wales. The fish were transported to Birmingham and maintained as described by Metcalfe & Butler (1982). Fish were divided into three experimental groups: control fish, those treated with alpha-methyl tyrosine, and sham-treated fish.

Fish were anaesthetized with tricaine methylsulphonate ($0{\cdot}04\,g\,l^{-1}$) (Sigma Chemical Co.) in unbuffered sea water, and placed ventral surface down on an operating table. The ventral aorta was cannulated with a length of polyethylene tubing (o.d. 1·22 mm) using the method described by Butler *et al.* (1986) and the dorsal aorta was cannulated with a similar length of polyethylene tubing (o.d. (1·00 mm) using the method described by Metcalfe & Butler (1982).

In those fish which were to receive either alpha-methyl tyrosine or a placebo (sham-treated fish), a third cannula, similar to that placed in the ventral aorta, was inserted into the peritoneum with the aid of a pointed stainless-steel wire through a small nick made in the ventral skin of the abdomen. All cannulae were secured tightly to the skin and the fish was placed in the experimental apparatus. This consisted of a large respirometer (volume 70 l) around which sea water was continuously circulated at a rate of about 20 l min^{-1} by a small pump. Sea water entered the respirometer from a gas exchange column into which aerated water flowed continuously at a rate of about 2 l min^{-1}. The partial pressure of O_2 in the water incurrent to the respirometer ($P_{inc}O_2$) was controlled by passing air or nitrogen through the gas exchange column at an appropriate rate. Water flowed out of the respirometer at a constant rate of between 600 and 800 ml min^{-1} which was measured with a flow meter (Meterate). At this flow, partial pressure of O_2 in water excurrent from the respirometer, ($P_{ex}O_2$) was normally maintained at about 2·7 kPa below $P_{inc}O_2$. The water level in the sealed respirometer remained constant, thus ensuring that incurrent and excurrent flows were equal.

Having been placed in the respirometer, the fish were allowed 5 days to recover from the surgical procedures and to acclimate to the experimental conditions. During this time, the treated fish received 50 mg kg^{-1} alpha-methyl tyrosine (Sigma Chemical Co.) per day for 5 days *via* the intraperitoneal cannula. The alpha-methyl tyrosine was dissolved in distilled water with 0·32 ml of 3 mol l^{-1} NaOH, and the solution was neutralized with 3 mol l^{-1} HCl just before administration (see Spector *et al.* 1965). Shame-treated fish received a daily dose of the carrier (as above) for 5 days. After 4 days of acclimation, the respirometer was sealed. 24 h later the respiratory frequency (fv) was measured by eye as the time taken for 100 breaths and calculated as breaths min^{-1}; $P_{inc}O_2$, $P_{ex}O_2$ and water flow through the respirometer were measured; and oxygen consumption ($\dot{M}_{O_2}$) calculated as described by Metcalfe & Butler (1988). Subsequently a small bung in the lid of the respirometer was briefly removed to allow access to the cannulae. These were drawn out through the respirometer lid and the bung quickly replaced, resealing the respirometer. This procedure had no measureable effect on the water P_{O_2} in the respirometer during either normoxia or hypoxia. The two arterial cannulae were connected to a blood pressure transducer (Druck, PDCR 75), the outputs from which were displayed on a pen recorder (Ormed) writing on rectilinear coordinates. Blood pressures in the ventral and dorsal aortae were measured and mean blood pressures calculated as described by Metcalfe & Butler (1982). Blood samples (about 0·8 ml) were drawn from the ventral and dorsal aortae for the measurement of partial pressures (P) and contents (C) of oxygen in mixed venous and arterial blood ($P\bar{v}_{O_2}$, Pa_{O_2}, $C\bar{v}_{O_2}$, Ca_{O_2}), respectively. A further blood sample (about 0·9 ml) was drawn from either the dorsal or ventral aorta; 100 μl of this sample was used to measure haematocrit and the remainder was used to determine the levels of circulating catecholamines by reverse-phase, ion-pair HPLC with electrochemical detection, as described by Butler *et al.* (1986). The P_{O_2} values of water and blood samples were measured with an oxygen electrode

(Radiometer) maintained at the experimental temperature. The output from the oxygen electrode was displayed on an acid–base analyser (Radiometer, PHM 71 Mk2). Since the water in the respirometer was continuously mixed, the P_{O_2} of the water excurrent to the respirometer was taken to be that of the water being inspired by the fish ($P_{I_{O_2}}$).

Subsequently the P_{O_2} of the water incurrent to the respirometer was reduced to between about 7·3 and 8·7 kPa by passing nitrogen through the gas exchange column at an appropriate rate. Initial tests showed that at the flows used, about 7 h was required for the P_{O_2} of incurrent and excurrent water to stabilize. Accordingly, fish were allowed at least 16–18 h to adjust to the hypoxic conditions before the values of the measured variables were recorded (as above).

The direct metabolic effect of alpha-methyl tyrosine on oxygen consumption in isolated dogfish hepatocytes

In the present study, alpha-methyl tyrosine markedly reduced oxygen consumption in intact normoxic dogfish (see Results). Although alpha-methyl tyrosine is reported not to affect carbohydrate metabolism in mammals (see Brogden *et al.* 1981), the possibility remained that alpha-methyl tyrosine may have had a direct depressive effect on cellular metabolism in elasmobranchs. This was investigated by examining the effect of alpha-methyl tyrosine on oxygen consumption in isolated dogfish hepatocytes, since liver cells have become widely used in cell metabolic research. This part of the study was intended to be a brief attempt to identify whether there was any evidence that alpha-methyl tyrosine was able to depress cellular metabolism.

Five dogfish which had been obtained and maintained as described above were used in this part of the study. Each fish was pithed and placed ventral side up on an operating table in a constant-temperature room maintained at 15 °C. The visceral cavity was opened and the hepatic portal vein cannulated with a length of 2 mm o.d. polyvinylchloride tube connected to the outflow from a pulsatile pump (Metcalfe & Butler, 1982) which had previously been filled with Hepes-buffered, Ca^{2+}-free dogfish saline containing 0·5 mmol l^{-1} EDTA and heparin (25 units ml^{-1} i.u., Weddel). The heart was punctured and perfusion was commenced *in situ* at a rate of about 10 ml min^{-1}. The liver was then removed intact from the fish, with the cannula in place, and placed in a large Petri dish. After 10–15 min and when all the blood and been washed from the liver, the perfusion medium was changed to Hepes-buffered saline (as above) containing 1·7 mmol l^{-1} $CaCl_2$ and 0·06 % (w/v) collagenase (Worthington) in a total volume of about 50 ml. This was recirculated through the liver at the above rate for about 1 h to soften the liver tissue and make it malleable. The liver was subsequently broken up and nonhepatic matter and remaining connective tissue removed by filtration through fine nylon netting. The remaining suspension was centrifuged at 100 *g* for 5 min to precipitate the hepatocytes and the supernatant was discarded. The pelleted cells were resuspended in Hepes-buffered saline and then recentrifuged (as above). This washing procedure was repeated two or three times until the supernatant was clear. All

purification steps were performed at 2–4°C on ice. Aerobic cellular respiration was measured using a polarographic oxygen electrode in a thermostatically controlled (at 15°C) cuvette which had a volume of about 2 ml (Rank Brothers, Cambridge), the contents of which were continuously stirred. The output from the oxygen electrode was displayed on a chart recorder (JJ Instruments Ltd).

Procedure

The cuvette was filled with approximately 2 ml of Hepes-buffered saline and the residual oxygen consumption of the electrode was recorded from the change in the P_{O_2} with time. 200 μl of hepatocyte suspension was added to the cuvette and, once stability had been reached, oxygen consumption was again recorded. Subsequently 0·5 mg of alpha-methyl tyrosine in a volume of 100 μl was added, achieving a concentration of about 0·25 mg ml^{-1}, and the oxygen consumption was recorded again. This concentration would be similar to the maximum that would have been reached *in vivo* after 5 days (250 mg kg^{-1}), assuming a uniform distribution of the drug among different tissues and a mean specific gravity of 1 for dogfish tissue.

The condition of the hepatocytes was ascertained by the addition of 5 mmol l^{-1} sodium succinate. The ratio of succinate-stimulated oxygen consumption to endogenous oxygen consumption, called the succinate stimulation index (SSI), has been shown to be a measure of cellular damage (Seibert, 1985). If the SSI was greater than 1·3, the results were discarded (one case only).

The oxygen electrode/cuvette was calibrated after each addition by injecting 100 μl of distilled water equilibrated with oxygen at 0°C. This contains 218·4 nmol l^{-1}. Oxygen consumption rates were calculated per milligram of protein after correction for the residual oxygen consumption of the electrode.

Samples of each hepatocyte preparation were retained and stored at −20°C for no longer than 5 weeks before analysis of the protein content using the method of Lowry *et al.* (1951) as modified by Hartree (1972).

Mean values are expressed ± the S.E. of the mean. Mean normoxic and hypoxic values within each group of animals have been compared using the paired *t*-test (Bailey, 1981). Comparisons among groups of animals have been made using Student's *t*-test. The term 'significant' refers to the 95 % level of confidence ($P < 0·05$).

Results

Series 1. Effects of alpha-methyl tyrosine on intact dogfish

Untreated fish (group 1), the response to hypoxia

In untreated, intact dogfish there were significant decreases in both pre- and postbranchial blood P_{O_2} and oxygen contents in response to hypoxia as the environmental P_{O_2} was reduced from 16·9 to 7·3 kPa (Table 1). Total oxygen consumption was significantly reduced to about 64 % of the normoxic value, even though there was a significant increase in respiratory frequency of 20 %. This level

Table 1. *Mean ± S.E. of the mean values of the measured variables from control, alpha-methyl-tyrosine-treated and sham-treated dogfish at 15°C during normoxia and in response to hypoxia*

	Control fish (group 1)			Alpha-methyl-tyrosine-treated-fish (group 2)			Sham-treated fish (group 3)		
	Normoxia	Hypoxia	*N*	Normoxia	Hypoxia	*N*	Normoxia	Hypoxia	*N*
$P_{I_{O_2}}$ (kPa)	16·92 ± 0·45	7·36 ± 0·68*	9	18·52 ± 0·31†	9·01 ± 0·76*	8	18·15 ± 0·20†	7·95 ± 0·31*	8
Pa_{O_2} (kPa)	10·46 ± 0·49	3·16 ± 0·47*	9	8·48 ± 1·27‡	4·45 ± 0·72	7	12·10 ± 0·61	3·96 ± 0·48*	6
$P\bar{v}_{O_2}$ (kPa)	2·00 ± 0·28	0·84 ± 0·12*	3	2·83 ± 0·27	1·47 ± 0·12*†	5	2·75 ± 0·47	1·05 ± 0·20*	7
Ca_{O_2} (mmol l^{-1})	2·42 ± 0·25	1·34 ± 0·21*	9	2·28 ± 0·10	1·70 ± 0·18*	7	1·82 ± 0·29	1·20 ± 0·28*	6
$C\bar{v}_{O_2}$ (mmol l^{-1})	0·94 ± 0·11	0·43 ± 0·06*	3	1·45 ± 0·09†	0·83 ± 0·12*†‡	5	0·99 ± 0·23	0·43 ± 0·10*	7
VAbp (kPa)	3·62 ± 0·57	3·52 ± 0·21	3	3·36 ± 0·19	3·06 ± 0·09	5	3·31 ± 0·38	2·63 ± 0·63	4
DAbp (kPa)	2·69 ± 0·21	1·89 ± 0·33*	8	2·34 ± 0·16	2·01 ± 0·18	6	2·65 ± 0·18	2·36 ± 0·12*	5
fv (breaths min^{-1})	50·6 ± 2·10	60·8 ± 3·10*	9	44·9 ± 4·20	62·2 ± 3·10*	8	46·4 ± 2·40	63·4 ± 2·60*	8
Haematocrit (%)	15·6 ± 1·51	14·3 ± 1·34	9	17·3 ± 0·90	16·5 ± 1·26	8	13·4 ± 1·53	13·6 ± 1·75	8
$\dot{M}_{O_2}$ (μmol min^{-1} kg^{-1})	35·46 ± 3·85	22·86 ± 1·99*	9	26·75 ± 4·13	20·74 ± 1·18	8	32·75 ± 1·58	24·79 ± 3·05*	8
NA (nmol l^{-1})	46 ± 16	387 ± 40*	9	12 ± 5	83 ± 46†	7	25 ± 13	278 ± 106	5
A (nmol l^{-1})	62 ± 22	212 ± 120	9	12 ± 4†	24 ± 10	7	13 ± 5	240 ± 129	5

* Signifies significant ($P < 0·05$) differences in response to hypoxia.

† Signifies significant ($P < 0·05$) differences between control fish and either alpha-methyl-tyrosine- or sham-treated fish.

‡ Signifies significant ($P < 0·05$) differences between alpha-methyl-tyrosine- and sham-treated fish.

$P_{I_{O_2}}$, partial pressure of O_2 in inspired water; Pa_{O_2}, partial pressure of O_2 in arterial blood; $P\bar{v}_{O_2}$, partial pressure of O_2 in mixed venous blood; Ca_{O_2}, concentration of O_2 in arterial blood; $C\bar{v}_{O_2}$, concentration of oxygen in mixed venous blood; VAbp, ventral aortic blood pressure; DAbp, dorsal aortic blood pressure; fv, rate of ventilation; $\dot{M}_{O_2}$, rate of oxygen consumption; NA, noradrenaline; A, adrenaline.

of hypoxia caused a significant bradycardia with heart rate decreasing from $39{\cdot}4 \pm 1{\cdot}8$ to $29{\cdot}2 \pm 2{\cdot}8$ beats min^{-1}. Prebranchial blood pressure remained unchanged in response to hypoxia but postbranchial blood pressure was significantly reduced. There was a significant (8·4-fold) increase in the level of circulating noradrenaline, although there was a less marked (3·4-fold) and insignificant increase in the circulating levels of adrenaline.

Treated fish (group 2)

In dogfish treated for 5 days with a daily intraperitoneal dose of 50 mg kg^{-1} alpha-methyl tyrosine, there was a reduction in the circulating levels of both adrenaline and noradrenaline to below those measured in either the control or the sham-treated animals, although it was only adrenaline that was significantly lower than in control fish. The only cardiovascular variables to be significantly different from either the control (group 1) or sham-treated (group 3) fish were Pa_{O_2} and mixed venous blood oxygen content. Pa_{O_2} in fish treated with alpha-methyl tyrosine was significantly lower than that in the sham-treated animals, whereas venous blood oxygen content was significantly higher than that of control fish. Despite the lower Pa_{O_2} in fish treated with alpha-methyl tyrosine, oxygen content of postbranchial blood was similar to those of both control and sham-treated fish, probably as a consequence of the higher haematocrit in fish treated with alpha-methyl tyrosine. Although the difference was not significant, it is important to note that the resting normoxic oxygen consumption in dogfish treated with alpha-methyl tyrosine was only 75 % of that of control fish and 82 % of that of sham-treated fish, despite very similar values for postbranchial blood oxygen content.

Although there were increases in the circulating levels of both adrenaline (twofold) and noradrenaline (6·9-fold) in response to hypoxia in fish treated with alpha-methyl tyrosine, these increases were not significant, and the absolute levels in hypoxia were much lower than those in either control or sham-treated fish. In fact, the hypoxic levels of both these hormones in treated fish were not significantly different from the normoxic values in either of the other two groups of fish, and the level of noradrenaline in hypoxic, treated fish was significantly lower than in control fish. As the environmental P_{O_2} decreased from 18·53 kPa to about 9·0 kPa, P_{O_2} and O_2 content of pre- and postbranchial blood decreased, although the decrease in Pa_{O_2} was not significant, and $P\bar{v}_{O_2}$ and prebranchial blood oxygen content did not fall to levels as low as those in either control or sham-treated fish. Pv_{O_2} in fish treated with hypoxia and alpha-methyl tyrosine was significantly higher than that in control fish, and prebranchial blood oxygen content was significantly higher than those in both control and sham-treated fish. However, these differences may be due to the higher hypoxic environmental P_{O_2} in the fish treated with alpha-methyl tyrosine. Oxygen consumption decreased only slightly in response to hypoxia to 78 % of the normoxic value and this decrease was not significant. However, there was a large (39 %) and significant increase in ventilatory frequency in response to hypoxia. The small decrease in postbranchial blood pressure was not significant.

Sham-treated fish (group 3)

Apart from the slightly higher environmental P_{O_2}, none of the variables measured in normoxia in the sham-treated fish was significantly different from those of control fish. The response to hypoxia was essentially similar to that seen in the control fish with a significant decrease in P_{O_2} and oxygen content of arterial and mixed venous blood, $\dot{M}_{O_2}$, and postbranchial blood pressure, and a significant increase in ventilatory frequency. Again there were large increases in the circulating levels of noradrenaline (11·1-fold) and adrenaline (18·5-fold), although these increases were not significant.

Series 2. The effects of alpha-methyl tyrosine on oxygen uptake in isolated hepatocytes

In view of the observation that normoxic oxygen consumption in fish treated with alpha-methyl tyrosine was substantially reduced compared with both control and sham-treated fish, despite similar postbranchial blood oxygen contents, it seemed possible that alpha-methyl tyrosine might have been affecting the resting metabolic rate in a way that reduced the fish's total demand for oxygen. This seemed all the more likely in view of the observation that venous blood returning to the heart from the systemic circulation had an oxygen content greater than that in either control or sham-treated fish. It was for these reasons that an attempt was made to estimate any direct depressive effect of alpha-methyl tyrosine on O_2 uptake in isolated hepatocytes.

In five hepatocyte samples containing 6–12 mg of protein, taken from different fish, a mean oxygen consumption rate of $35{\cdot}6 \pm 6{\cdot}1$ nmol O_2 mg^{-1} h^{-1} was obtained. Addition of 0·5 mg of alpha-methyl tyrosine (equivalent to the total accumulated dose received by the intact fish) significantly reduced the mean oxygen consumption rate by $39 \pm 10\%$ to $21{\cdot}2 \pm 4{\cdot}0$ nmol O_2 mg^{-1} h^{-1}, indicating a significant depressive effect of alpha-methyl tyrosine on cellular O_2 consumption.

Discussion

The response to hypoxia (untreated fish)

Qualitatively, the cardiovascular and respiratory responses to hypoxia in the present study were similar to those previously reported for this species at similar temperatures (Butler & Taylor, 1971, 1975; Short *et al.* 1979; Metcalfe & Butler, 1984*a*). As environmental P_{O_2} decreased, there was a significant increase in fv and a reduction in heart rate. P_{O_2} and the O_2 content of arterial and mixed venous blood were all reduced and this was associated with significant reductions in oxygen consumption ($\dot{M}_{O_2}$).

The effects of catecholamine depletion with alpha-methyl tyrosine

In normoxic fish treated with alpha-methyl tyrosine, circulating catecholamine

levels were lower than those in either control or sham-treated fish and, although these did increase in response to hypoxia, the levels reached were far below those measured in either control or sham-treated fish during hypoxia and were not significantly different from the normoxic levels either within the group, or between control or sham-treated fish. Thus, it appears that alpha-methyl tyrosine does inhibit catecholamine synthesis in dogfish and is indeed effective in preventing the large increase in circulating levels in response to hypoxia.

In addition to its effects on circulating catecholamine levels, alpha-methyl tyrosine also tended to reduce normoxic Pa_{O_2} and $\dot{M}_{O_2}$ compared with both control and sham-treated fish. However, two observations suggest that the reduced $\dot{M}_{O_2}$ is not a consequence of the lower Pa_{O_2}. First, postbranchial blood O_2 content was similar to that in control fish, and actually higher than that in sham-treated fish. Second, if $\dot{M}_{O_2}$ was limited by the oxygen supply to the systemic circulation, $P\bar{v}_{O_2}$ might have been expected to be low, yet $P\bar{v}_{O_2}$ in fish treated with alpha-methyl tyrosine was greater than in either control or sham-treated fish, although not significantly so. It appears therefore that the reduced normoxic $\dot{M}_{O_2}$ in fish treated with alpha-methyl tyrosine is not a consequence of any cardiovascular effect, but is possibly due to some metabolic effect, even though alpha-methyl tyrosine is reported not to affect carbohydrate metabolism, at least in mammals (see Brogden *et al.* 1981).

The response of dogfish treated with alpha-methyl tyrosine to hypoxia was, in most respects, similar to that of both sham-treated and control fish. However, the most striking difference between treated fish and the two control groups was the small and insignificant decrease in $\dot{M}_{O_2}$ such that the hypoxic $\dot{M}_{O_2}$ was similar in all three groups of animals. The lack of a significant decrease in $\dot{M}_{O_2}$ in fish treated with alpha-methyl tyrosine in response to hypoxia appears to be due to a combination of the already low normoxic level of oxygen consumption and the relatively high venous blood oxygen content. Nonetheless, it is curious that $\dot{M}_{O_2}$ should decrease at all in these animals in response to hypoxia when venous blood O_2 content does not seem to be limiting.

Despite the effects of alpha-methyl tyrosine on normoxic oxygen consumption, neither blood oxygen content nor $\dot{M}_{O_2}$ was significantly lower than in either control or sham-treated fish during hypoxia, indicating that the lack of a large increase in the levels of circulating catecholamines in these treated fish does not compromise their oxygen uptake ability during environmental hypoxia.

In a previous study in which we attempted to assess the role of the increase in the circulating levels of catecholamines in response to hypoxia (Metcalfe & Butler, 1988), we found that pharmacological blockade of alpha- and beta-adrenergic receptors did not reduce either oxygen consumption or the effectiveness of the gills in transferring oxygen (measured as $\dot{T}_{O_2}$; Randall *et al.* 1967) either in normoxia or hypoxia. From both studies, it appears that the large increase in the circulating levels of catecholamines that occurs in response to hypoxia in the dogfish is not involved in enhancing oxygen uptake. What then is the role of this increase? Dogfish are able to withstand quite low environmental oxygen levels for

extended periods (Butler *et al.* 1979); presumably this is achieved by combining a reduction in total metabolism, as indicated by the reduction in activity in response to hypoxia (Metcalfe & Butler, 1984*a*), with a partial switch to anaerobiosis (Butler *et al.* 1979). Anaerobiosis yields much less energy per molecule of substrate than does aerobiosis, so larger amounts of substrate may have to be mobilized in response to hypoxia. This is perhaps why the increase in circulating levels of catecholamines is important, since DeRoos & DeRoos (1978) have shown that these hormones elevate plasma glucose levels when injected into intact elasmobranchs. In a previous study, Butler *et al.* (1979) showed that at the end of prolonged hypoxia (72 h), plasma glucose levels were not elevated above normoxic control levels. However at 72 h, plasma catecholamine levels had declined to only 2–3 times resting levels from a level of more than 10 times resting levels at 1·5 h of hypoxia (Butler *et al.* 1978).

The effects of alpha-methyl tyrosine on oxygen uptake in isolated dogfish hepatocytes (series 2)

The resting oxygen consumption rates of isolated dogfish hepatocytes measured in the present study are generally lower than those taken from the liver of either trout (Hazel & Sellner, 1979; Seibert, 1985) or eels (Jankowsky *et al.* 1984), even when the slightly lower temperature used in the present study (15°C *vs* 20°C) is taken into account. However, the present values are similar to those reported by Moerland & Sidell (1981) for the killifish *Fundulus heteroclitus*.

The addition of alpha-methyl tyrosine to the hepatocyte sample to achieve a concentration similar to the total accumulated dose given to intact dogfish (250 mg kg^{-1}) caused a 39 % decrease in $\dot{M}_{O_2}$. However, in intact fish treated with alpha-methyl tyrosine, $\dot{M}_{O_2}$ was 18% and 25 % lower than in sham-treated and control fish, respectively. Consequently, although this part of the study has only involved a very preliminary investigation of the metabolic effects of alpha-methyl tyrosine in elasmobranchs, it appears that the reduced normoxic $\dot{M}_{O_2}$ observed in dogfish treated with alpha-methyl tyrosine may have been due to the direct effect of this drug on cellular metabolism, rather than to any effect on the circulating levels of catecholamines. It is unfortunate that although alpha-methyl tyrosine is very useful for inhibiting the synthesis of catecholamines in elasmobranchs, the metabolic effects of the drug limit its usefulness in studies of oxygen uptake.

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