

Review

The adrenergic stress response in fish: control of catecholamine storage and release

Stephen G. Reid *, Nicholas J. Bernier, Steve F. Perry

Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario K1N 6N5, Canada

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Abstract

In fish, the catecholamine hormones adrenaline and noradrenaline are released into the circulation, from chromaffin cells, during numerous 'stressful' situations. The physiological and biochemical actions of these hormones (the efferent adrenergic response) have been the focus of numerous investigations over the past several decades. However, until recently, few studies have examined aspects involved in controlling/modulating catecholamine storage and release in fish. This review provides a detailed account of the afferent limb of the adrenergic response in fish, from the biosynthesis of catecholamines to the exocytotic release of these hormones from the chromaffin cells. The emphasis is on three particular topics: (1) catecholamine biosynthesis and storage within the chromaffin cells including the different types of chromaffin cells and their varying arrangement amongst species; (2) situations eliciting the secretion of catecholamines (e.g. hypoxia, hypercapnia, chasing); (3) cholinergic and non-cholinergic (i.e. serotonin, adrenocorticotrophic hormone, angiotensin, adenosine) control of catecholamine secretion. As such, this review will demonstrate that the control of catecholamine storage and release in fish chromaffin cells is a complex processes involving regulation via numerous hormones, neurotransmitters and second messenger systems. © 1998 Elsevier Science Inc. All rights reserved.

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1. Introduction

The catecholamine hormones, adrenaline and noradrenaline, are released from chromaffin cells, into the circulation of fish, during exposure to a wide range of internal and environmental stressors [176,201,202,251]. Once in the circulation, these hormones function to diminish the plethora of detrimental consequences often associated with stressful situations. In particular, one of the primary roles of plasma catecholamines is to modulate cardiovascular and respiratory function in order to maintain adequate levels of oxygen in the blood and, therefore, a sufficient supply to the tissues. In addition,

these biogenic amines serve to mobilize energy stores to provide for the increased energy demands that often accompany stress [231,235]. Given the multitude of effects exerted by these hormones, both the physiological and biochemical consequences of an elevation of plasma catecholamines have formed an extensive field of investigation by fish physiologists and biochemists over the past several decades. However, equally important to the general understanding of the physiology of catecholamines are the mechanisms underlying their release from the chromaffin cells into the circulation. In comparison with the literature regarding the consequences of catecholamine release, little is known about the release process of these hormones in fish. On the other hand, the control of catecholamine release is an area of extensive study in mammalian physiology (see reviews from the Symposium on the Adrenal Gland;

* Corresponding author. Present address: Department of Biology, McMaster University, 1280 Main St. W., Hamilton, Ontario, L8S 4K1, Canada. Tel.: +1 905 5259140, ext. 27412; fax: +1 905 5226066; e-mail: reidsg@mcmaster.ca

[39,75,144,167,217,250,254,273,277,293]). Indeed, in mammals, the chromaffin cell is an important model used in the study of secretion and exocytosis. As such, the goals of this review are to provide a detailed account of the afferent limb of the adrenergic stress response from catecholamine biosynthesis and storage through events culminating in the secretion of catecholamines from the chromaffin cells, into the circulation.

2. Storage of catecholamines

2.1. Catecholamine biosynthesis

Catecholamines, adrenaline and noradrenaline, are synthesized, within the chromaffin cell, via the Blaschko pathway beginning with the amino acid precursor tyrosine [30,122]. Both the catecholamine biosynthetic pathway and the metabolic processes resulting in their degradation, in fish, have recently been reviewed [235]. In this current paper, a general account of catecholamine biosynthesis is discussed in addition to a variety of factors that have been implicated in modulating biosynthesis in various vertebrate species. The initial step in biosynthesis is the hydroxylation of tyrosine by the enzyme tyrosine hydroxylase (TH) to form L-dihydroxyphenylalanine (L-DOPA) in the rate-limiting step in the synthesis of noradrenaline. Tyrosine hydroxylase has an absolute requirement for both Fe^{2+} and molecular oxygen while tetrahydropteridine serves as a cofactor. In the Atlantic cod (*Gadus morhua*), the pH and temperature optima of this enzyme are 6.0 and 30–35°C, respectively [136].

A variety of factors are capable of regulating the activity of TH in various vertebrate species [131]. First, TH activity is subject to end-product inhibition by dopamine and noradrenaline suggesting a rapid and specific control of the rate of catecholamine biosynthesis in vivo [255]. Additionally, a variety of neuronal and hormonal factors are capable of increasing the activity of TH. Some of these include, increased nervous stimulation of the chromaffin cells [185,186,264,265], adrenocorticotrophic hormone (ACTH) within the blood [187], nicotine [119,258] and glucocorticoids [258]. Additionally, a variety of neuropeptides including vasoactive intestinal polypeptide (VIP), pituitary adenylyl cyclase activating polypeptide (PACAP), secretin and angiotensin [285,290,308] have all been demonstrated to influence TH activity.

The second step in catecholamine biosynthesis involves the enzyme L-aromatic amino acid decarboxylase (AADC, or DOPA decarboxylase) which decarboxylates L-DOPA to form dopamine. This enzyme has a broad substrate affinity and specificity and is present in sufficient quantities that L-DOPA does not accumulate

to any substantial extent [122]. AADC requires pyridoxal-5-phosphate as a cofactor and can be inhibited by compounds structurally similar to L-DOPA, such as α -methyl dopa and *N*-methylmelamide [179].

The hydroxylation and decarboxylation reactions catalyzed by TH and AADC, respectively, both occur within the cytosol. The end product of the AADC reaction, dopamine, is then transported into secretory vesicles (granules) where the enzyme dopamine- β -hydroxylase ($\text{D}\beta\text{H}$) converts it into noradrenaline via a side chain hydroxylation. $\text{D}\beta\text{H}$, a copper-containing tetrameric glycoprotein, requires both molecular oxygen and ascorbic acid as cofactors. $\text{D}\beta\text{H}$ is present in secretory granules in both a membrane bound form and a soluble form [135], the ratio of which differs amongst species [63]. A portion of the soluble fraction of $\text{D}\beta\text{H}$ may be released, together with the catecholamines, ATP and chromogranins, during exocytosis [37]. The temperature optima for $\text{D}\beta\text{H}$ in the dogfish and Atlantic cod are 24.5–32 and 27°C, respectively while the pH optima are 5.4–6.2 and 5.4, respectively [132,134].

The activity of $\text{D}\beta\text{H}$ in fish chromaffin tissue has been documented in numerous studies of a variety of fish species including Atlantic cod, *Gadus morhua* [134], spotted gar, *Lepisosteus platyrhincus* [6], African lungfish, *Protopterus aethiopicus* [4], two elasmobranchs, *Squalus acanthias* and *Etmopterus spinax* [132] and the Atlantic hagfish, *Myxine glutinosa* [133]. Most tissues, in mammals, possess endogenous inhibitors of $\text{D}\beta\text{H}$, such as sulfhydryl compounds, that form complexes with the Cu^{2+} at the active site of the enzyme. These inhibitors can be inactivated by the addition of Cu^{2+} or *N*-ethylmaleimide, a sulfhydryl blocker, a necessary procedure when measuring the in vitro activity of this enzyme [24]. Like tyrosine hydroxylase, $\text{D}\beta\text{H}$ in mammals is also subject to neuronal regulation where increased nervous stimulation of the chromaffin cell results in an increase in $\text{D}\beta\text{H}$ activity [55]. Additionally, hormonal regulation of $\text{D}\beta\text{H}$ via adrenocorticotrophic hormone and glucocorticoids also functions to modulate the activity of this enzyme [289].

In some chromaffin cells (see Section 2.2), noradrenaline is transported from the secretory vesicle, into the cytoplasm, where it is methylated to form adrenaline by the enzyme phenylethanolamine-*N*-methyltransferase (PNMT). It is not known how noradrenaline is made available to cytoplasmic PNMT nor how, once synthesized in the cytosol, adrenaline is transported into the storage vesicles. Alternately, once synthesized, noradrenaline may remain stored within the secretory granules. PNMT activity has also been measured in a variety of fish species including the Atlantic hagfish, *M. glutinosa* [133], the spotted gar, *L. platyrhincus* [6], the African lungfish, *Rotopterus aethiopicus* [4], the rainbow trout, *Oncorhynchus mykiss* [175,241], the Atlantic cod, *G. morhua* [3,7] and the

dogfish, *S. acanthias* [2]. A comparison of optimal activities of TH, D β H and PNMT suggests that the conversion of noradrenaline to adrenaline (PNMT reaction) is the rate-limiting step in adrenaline formation [131].

PNMT is inhibited by its end product adrenaline, and to a lesser extent by noradrenaline [95–97]. Furthermore, the inhibition occurs at concentrations of adrenaline and/or noradrenaline normally present in adrenal medullary chromaffin cells suggesting a specific physiological role, particularly for adrenaline, in regulating PNMT activity. As with TH and D β H, both nervous (cholinergic) stimulation of chromaffin cells [84] and glucocorticoids [248,291,300,301] have been demonstrated to influence PNMT activity in mammals. However, modulation of PNMT activity by glucocorticoids does not appear to occur in fish or other lower vertebrates (see Section 2.5).

2.2. Arrangement of chromaffin cells

Chromaffin cells originate embryologically from the neural crest [58,62,160], and derive their name from the granulated brownish-yellow appearance, produced by the oxidation of intracellular catechols or indolamines, following staining with chromate and/or dichromate [58,147]. The arrangement of the catecholamine-containing chromaffin cells differs across the entire vertebrate lineage. In mammals, chromaffin cells are found within the adrenal gland, specifically the adrenal medulla, surrounded by the adrenal cortex that contains the steroidogenic interrenal cells [60,62,201]. In birds, chromaffin cells are also located within the adrenal gland but, in avian species, there is no division into medulla or cortex and the chromaffin and interrenal cells are intermingled [58,114]. Chromaffin cells in reptiles form a similar arrangement to those in birds [298,299]. In urodele amphibians, chromaffin cells are located within segmented bodies associated with sympathetic nerves on the ventral side of the kidney whilst, in anurans, chromaffin cells form a distinct mass on the surface of the kidney [8–13,58,60,108,109].

Amongst different classes of fish, the arrangement of chromaffin cells also exhibits substantial diversity. In evolutionary terms, the cyclostomes/agnathans (i.e. hagfish and lampreys) represent the most primitive group of fish. In these fish, the chromaffin cells are distributed within the systemic and portal hearts [15,32,33,130,212,213,281] and in large veins and arteries [15,127,128]. Elasmobranchs represent a more advanced evolutionary stage than the cyclostomes and, in this group, the chromaffin cells are associated with paravertebral autonomic ganglia [2,170,252,253,281]. The axillary bodies, comprising chromaffin cells in association with the gastric ganglia, are the primary source of circulating catecholamines in these fish [201,202]. In

dogfish, *S. acanthias* [2]. A comparison of optimal activities of TH, D β H and PNMT suggests that the conversion of noradrenaline to adrenaline (PNMT reaction) is the rate-limiting step in adrenaline formation [131].

The most evolutionary advanced fish are represented by the teleosts. Initial descriptions of the chromaffin cell, in teleosts, as the homologue of the adrenal gland, were documented in the early 20th century ([103,104,106,107], also see review [58]). In teleost fish, the chromaffin cells are located within the walls of the posterior cardinal vein (PCV) and in close association with the lymphoid tissue of the kidneys, particularly in the anterior region of the kidney ('head kidney') ([100,115,172,173,201,202,238,239,305]; Fig. 1A, B). In general, chromaffin cells are often observed either singly or clustered into groups of several cells. The association of chromaffin cells with the steroidogenic interrenal cells may vary amongst different teleost species [58,189].

2.3. Different chromaffin cell types

The primary circulating catecholamines in fish are adrenaline and noradrenaline with only a few studies having measured levels of circulating dopamine [42,78,79,115]. A variety of studies have demonstrated that adrenaline and noradrenaline are stored, at least in part, in separate populations of chromaffin cells.

The presence of both noradrenaline- and adrenaline-containing chromaffin cells within the posterior cardinal vein and head kidney has been demonstrated in the freshwater cyprinid, *Scardinius erythrophthalmus* [172]. The two different cell types were distinguished on the basis of light microscopy studies and of morphological characteristics at the electron microscope level. Noradrenaline-containing cells had strongly electron-dense secretory granules [59,61] that were generally spherical in shape. The enhanced electron density of noradrenaline containing granules arises from the precipitation of noradrenaline following fixation with glutaraldehyde. Adrenaline, on the other hand, remains soluble following glutaraldehyde fixation such that, following postfixation staining with dichromate, noradrenaline-containing cells appear more electron dense than adrenaline-containing cells. Granules within the noradrenaline-containing cells were spherical in appearance (average diameter 200 nm) and distributed evenly throughout the cell with the exception of the region of the nucleus. In the adrenaline-containing cells, granules were spherical or elongated (diameter 30 nm), and also were distributed homogeneously throughout the cell [172]. Other cell types were observed which had a density midway between the noradrenaline- and adrenaline-containing cells and in some cells the granules appeared empty. These later cell types may represent a different stage of chromaffin cell development or contain amines other than the catecholamines.

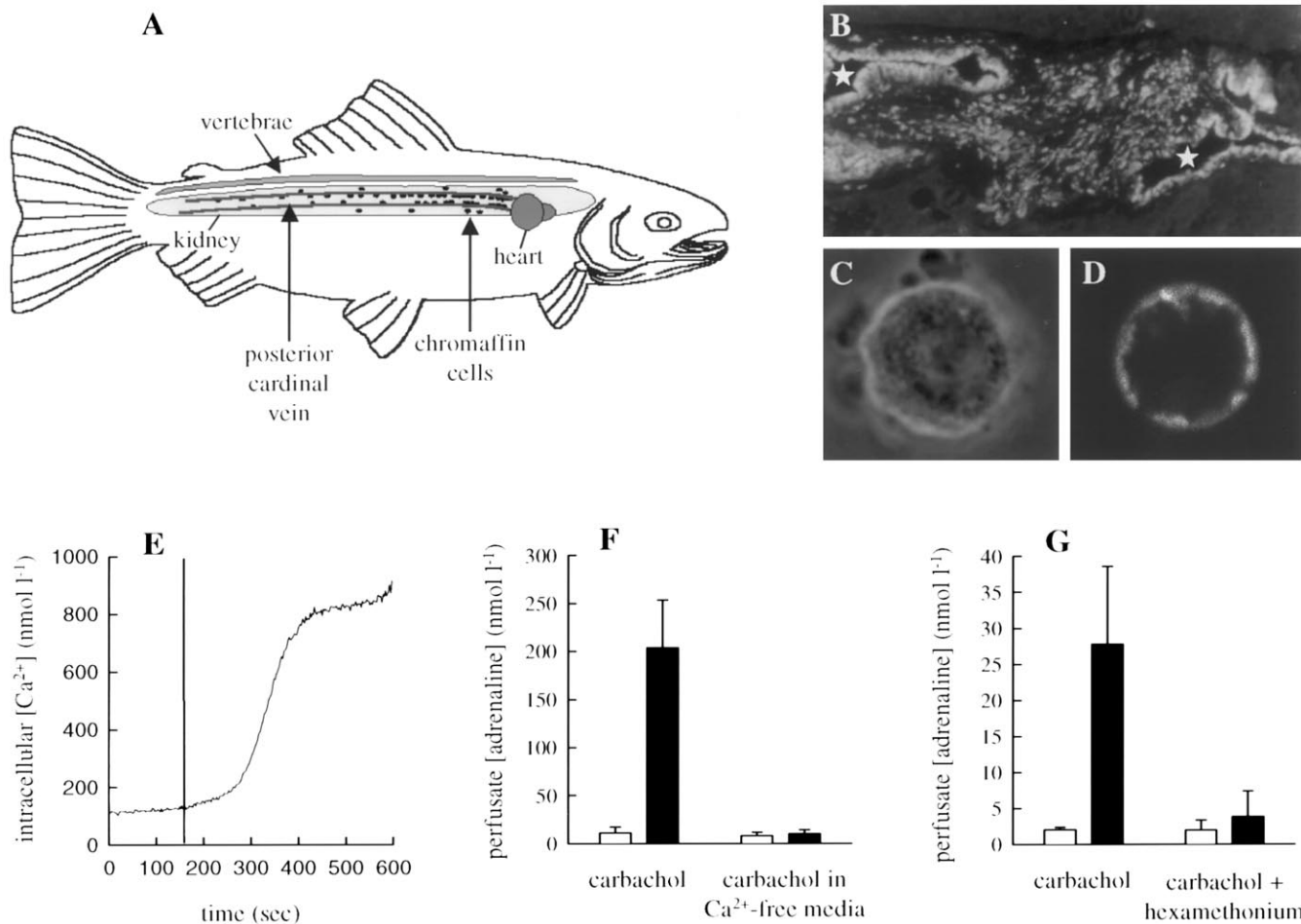


Fig. 1. The location, appearance and pharmacological properties of teleost fish chromaffin cells. (A) Chromaffin cells in teleost fish (a rainbow trout in the schematic) are located in the head kidney region within the walls of the posterior cardinal vein as it runs through the kidney to the heart. (B) A sagittal section through the head kidney/posterior cardinal vein in the Atlantic cod, *G. morhua* (magnification $\times 90$). An asterisk (*) denotes the lumen of the PCV. Chromaffin cells are labeled (white) with a fluorescent antibody raised against dopamine- β -hydroxylase, the enzyme that converts dopamine into noradrenaline. (C) A phase contrast view of an individual chromaffin cell (magnification $\times 2000$). Note the granular appearance of the cell indicative of secretory granules. (D) Labeling of nicotinic cholinergic receptors on a single chromaffin cell with fluorescently-tagged α -bungarotoxin (magnification $\times 2000$). (E) The temporal changes in intracellular Ca^{2+} concentration $[Ca^{2+}]$ in a single rainbow trout chromaffin cell after the addition of nicotine (at the vertical line). (F) The release of adrenaline, in situ, in response to the mixed muscarinic/nicotinic agonist carbachol carbamylcholine is abolished in Ca^{2+} -free media (data from rainbow trout). Open bars indicate perfusate adrenaline levels prior to the addition of carbachol; solid bars represent values after carbachol addition. (G) Pre-treatment with the ganglionic blocker hexamethonium, to block nicotinic receptors, also eliminates carbachol-evoked catecholamine release (data from the American eel, see text for eel-trout differences). Open bars indicate perfusate adrenaline levels prior to the addition of carbachol; solid bars represent values after carbachol addition.

The presence of these two cell types has also been demonstrated in the head kidney of the stickleback, *Gasterosteus aculeatus* [100] and the carp, *Cyprinus carpio* [174]. In the Holostean fish, *Amia calva*, [307] only one population of secretory granules was observed based on electron-density, and as such, it was not possible to distinguish between adrenaline- and noradrenaline-containing cells in this species.

Utilizing light and electron microscopy, the presence of both noradrenaline- and adrenaline-containing chromaffin cells has been demonstrated in the rainbow trout [173]. Additionally, other immunohistochemical studies have revealed the presence of separate noradrenaline

and dopamine containing cells in the sea bass, *Dicentrarchus labrax* (L.) [1] and the American eel, *Anguilla rostrata* [117]. Recently, positive labeling reactions for antisera raised against tyrosine hydroxylase (TH) and dopamine- β -hydroxylase ($D\beta H$) have been observed in the rainbow trout, Atlantic cod (Fig. 1B) and European eel chromaffin cell [237]. Of these TH- and $D\beta H$ -positive cells, a sub-population also exhibited a positive labeling reaction for PNMT, the enzyme which catalyses the methylation of noradrenaline into adrenaline. This sub-population of PNMT-positive cells were considered to be adrenaline-containing cells whereas those which exhibited a positive labeling reaction for TH and

Table 1
The levels of adrenaline and noradrenaline ($\mu\text{g g}^{-1}$ tissue weight) stored within the chromaffin tissue of various species of fish (cyclostomes, dipnoans, elasmobranchs, ganoids and teleosts).

Species	Storage Site	Adrenaline	Noradrenaline	Reference
Cyclostomes				
<i>M. glutinosa</i>	Kidney/great veins	<0.04	22	[281]
<i>M. glutinosa</i>	Systemic heart (atria)	8	18	[281]
<i>M. glutinosa</i>	Portal heart	3	51	[281]
<i>M. glutinosa</i>	Systemic heart	59	7	[281]
<i>M. glutinosa</i>	Systemic heart	20	20	[220]
<i>M. glutinosa</i>	PCV	2	50	[220]
<i>P. marinus</i>	Systemic atrium	90	3.4	[175]
<i>P. marinus</i>	Systemic ventricle	17	0.7	[175]
Dipnoans				
<i>P. aethiopicus</i>	Heart	4	71	[4]
<i>P. aethiopicus</i>	Intercostal arteries	216	94	[4]
Elasmobranchs				
<i>S. acanthias</i>	Sympathetic chain/axillary body	445	2139	[2]
<i>S. acanthias</i>	Sympathetic chain/axillary body	3100	6700	[281]
<i>C. monstrosa</i>	Axillary bodies	3780	9300	[232]
Ganoids				
<i>L. Platyrhincus</i>	Cardinal veins	48	22	[200]
<i>H. huso</i>	Cardinal veins	20	5	[20]
Teleosts				
<i>O. mykiss</i>	Head kidney	4.7	4.5	[188]
<i>O. mykiss</i>	PCV	110	30	[239]
<i>O. mykiss</i>	Kidney tissue	7	4	[239]
<i>A. rostrata</i>	Anterior kidney	84	42	[115]
<i>A. rostrata</i>	Anterior PCV	20	5	[239]
<i>G. callarius</i>	Head kidney	45	Undetectable	[281]
<i>G. morhua</i>	Cardinal veins	38	14	[7]
<i>C. carassius</i>	Head kidney	~0.05	0.16	[197]
<i>C. carpio</i>	Head kidney	~0.05	0.84	[257]

The particular region of tissue storage site (examined in each study reference) is indicated in the table. PCV, posterior cardinal vein. See text for additional details.

D β H, but not PNMT, were considered to be noradrenaline containing cells. Anecdotally, this phenomenon was more pronounced in cod and trout than in the eel [237].

Also in the rainbow trout, the differential release of adrenaline, over noradrenaline, in response to stimulation with a cholinergic agonist has been demonstrated utilizing an in situ saline-perfused posterior cardinal vein preparation [239]. In this preparation, perfusion with $60 \text{ mmol l}^{-1} \text{ K}^+$, a non-specific depolarizing stimuli, caused the exocytotic release of catecholamines by directly altering membrane potential without the intervention of receptors or receptor-coupled second messenger systems [37]. Perfusion with $60 \text{ mmol l}^{-1} \text{ K}^+$ did not alter the adrenaline:noradrenaline ratio in the perfusate, compared with ratio prior to stimulation. However, the application of the acetylcholine agonist, carbamylcholine (carbachol, 10^{-8} – $2 \times 10^{-7} \text{ mol}$) caused the adrenaline:noradrenaline ratio to increase from approximately 1 to 4–8. This preferential release of adrenaline over noradrenaline in response to carba-

chol, but not $60 \text{ mmol l}^{-1} \text{ K}^+$, is consistent with the presence of both noradrenaline- and adrenaline-containing cells with the adrenaline-containing cells having a greater sensitivity to cholinergic stimulation than the noradrenaline-containing cells. Similar trends were observed in the American eel.

2.4. Differences in storage levels

Both the concentration of catecholamines within the chromaffin tissue of various species as well as the prevalence of one catecholamine over the other can differ to a large extent within, and between, different classes of fish (Table 1) [235]. This is evident from an early study [281] that reports storage levels of adrenaline and noradrenaline in various organs from a cyclostome (*M. glutinosa*), an elasmobranch (*S. acanthias*) and a teleost (*G. callarius*).

An early study on *Myxine*, [281] reported substantially greater levels of noradrenaline than adrenaline in the kidney-great veins, the atria of the systemic heart

and in the entire portal heart whereas in the ventricle of the systemic heart, adrenaline was the primary catecholamine. A recent study, [220] reports equivalent levels of both catecholamines in the systemic heart of *Myxine* yet, in the posterior cardinal vein noradrenaline was dominant. In another cyclostome, *Petromyzon marinus* [175] the concentration of adrenaline was greater than noradrenaline in both the systemic atrium and ventricle. In the dipnoan (*Protopterus aethiopicus*), noradrenaline is predominant in the heart while adrenaline is the primary catecholamine stored in the intercostal arteries [4].

Within the axillary bodies/sympathetic chain ganglia in elasmobranchs, noradrenaline appears to be the predominant catecholamine. In the spiny dogfish, *S. acanthias*, several studies [2,281] observed greater quantities of noradrenaline than adrenaline in the sympathetic chain/axillary bodies [2,281]. Within the axillary bodies of another elasmobranch, *Chimarea monstrosa*, the concentration of noradrenaline was approximately 2.5 times that of adrenaline [232]. The concentration of stored catecholamines in elasmobranchs thus appears to be substantially larger than in other fish species. However, these enhanced storage levels do not appear to be manifested as larger levels of circulating catecholamines observed during stress. Indeed, during various stressful situations, levels of circulating catecholamines in teleosts appear, in general, to be greater than those in elasmobranchs [43,42,267]. Adrenaline is the predominant catecholamine within the cardinal veins in two species of ganoids *L. platyrhincus* [200] and *Huso huso* [20].

In teleost species there also exist various differences in the levels of stored catecholamines amongst species and even amongst the same species examined by different investigators. The extent to which these differences, within the same species, are due to differences in experimental approach is unknown. Unlike elasmobranchs where noradrenaline is the predominant catecholamine and cyclostomes where the prevalence of one catecholamine over the other is tissue specific, in teleost fish adrenaline is the primary stored catecholamine in most species studied to date. In rainbow trout, *O. mykiss*, one study [188] observed approximately equivalent levels of noradrenaline and adrenaline in the head kidney. However, other investigators [239] observed greater levels of adrenaline than noradrenaline in regions of the main branch of the posterior cardinal vein and in regions of kidney tissue. It is evident from these studies that the anterior two thirds of the posterior cardinal vein is the primary storage site for catecholamines with little contribution from the kidney tissue itself. As with the rainbow trout, the primary storage site for catecholamines in the American eel (*Anguilla rostrata*) is also the anterior region of the posterior cardinal vein and adrenaline is predominant over noradrenaline.

Again, differences in absolute levels of these hormone are found between studies and the tissue regions studied [115,239]. The reason for the differences between these two studies is unknown but it is evident that the concentration of stored adrenaline is two to four times greater than stored noradrenaline.

In the common cod (*G. callarius*), adrenaline is the exclusive catecholamine within the head kidney with virtually undetectable levels of noradrenaline [281]. In the Atlantic cod (*G. morhua*), [7] adrenaline is also found in greater quantities than noradrenaline in the cardinal veins. In *C. carassius* [197] and *C. carpio* [257] the levels of stored catecholamines are substantially reduced compared to the other teleost species reported.

It is evident that within fish species there is substantial variability in the absolute values of stored catecholamines and the ratio of adrenaline to noradrenaline as well as differences within the same species reported by various investigators. It would appear that in the cyclostomes and elasmobranchs, noradrenaline is the predominant catecholamine whilst in the teleosts (with the exception of the carp), adrenaline is predominant. It is possible that, in teleosts, the prevalence of adrenaline over noradrenaline represents an evolutionary alteration/up-regulation in the enzymatic processes required to synthesize adrenaline. It is plausible that in teleosts, compared with cyclostomes and elasmobranchs, (PNMT) has a greater activity/substrate affinity or is more resistant to degradation.

2.5. Modulation of storage levels

The acute adrenergic response in fish can be substantially modified by the prior history of the fish as well as environmental factors. Indeed, as mentioned above, a variety of factors (hormonal and neuronal) are capable of regulating and modulating the activity of the enzymes involved in biosynthesis. However, these hormonal and neuronal modulatory influences are generally 'expressed' only upon exposure of the animal (fish or otherwise) to various internal or external conditions which either stress the animal or necessitate an alteration in the capacity to express the adrenergic stress response. In fish, a variety of conditions such as anoxia, pollution, nutritive stress, physical stress and hormonal influences can all modify the levels of stored catecholamines in fish chromaffin tissue.

Recently, it was demonstrated that long-term exposure (76–160 h) of crucian carp, *C. carassius* L. to anoxic conditions produced a substantial decrease in stored noradrenaline levels within the head kidney [196]. Moreover, re-establishment of normoxic conditions resulted in a return to pre-anoxia storage levels after only 40 min suggesting an efficient synthesis pathway given the availability of molecular oxygen. Conversely, exposure of this same species to 17 days of

anoxia [197] produced a 92% decrease in stored noradrenaline levels which, upon re-exposure to normoxia, did not recover to pre-anoxia levels. In both studies [196,197], there were no effects of anoxia on stored adrenaline levels. It is possible [197] that the differences in the recovery of storage levels from anoxia in the two studies may reflect inactivation of $D\beta H$ given its requirement for molecular oxygen as a cofactor. It remains to be seen whether storage levels would recover if the animals were maintained under normoxic conditions for longer periods of time following the anoxic exposure. Presumably *de novo* synthesis of biosynthetic enzymes would permit the synthesis of catecholamines, given the necessary substrates, and recovery of storage levels.

Another environmental perturbation that can diminish levels of stored catecholamines in chromaffin tissue is exposure to various organic pollutants. Several industrial complexing agents, diethyldithiocarbamate (DOC) and amyloxanthate (AX) form complexes with heavy metals, increasing their availability to fish and compromising the animals health [31,275]. Exposure of rainbow trout to DOC for a period of 24 h caused a decrease in both adrenaline and noradrenaline storage levels whereas exposure to AX for 24 h resulted in a decrease in stored noradrenaline, but not adrenaline [198]. It was hypothesized [198] that the decrease in storage levels, following these treatments, is due to an inhibition of $D\beta H$ arising as a result of these agents complexing Cu^{2+} ions which are essential for normal $D\beta H$ function.

In addition to environmental perturbations such as anoxia and pollution, the nutritional status of the animal may also influence the levels of stored catecholamines. Depriving rainbow trout of food for a period of 2 months produced a decrease in the total content of catecholamines (μg of catecholamines) stored within the kidney and posterior cardinal vein, compared with normally fed fish [238]. The lower catecholamine content in these tissues is consistent with the diminished tissue weight of the kidney and PCV following food deprivation. Contrary to the decrease in total catecholamine content, the concentration of catecholamines (μg catecholamine g^{-1} tissue weight) within these tissues was unaffected by the starvation regimen. Tyrosine, the amino acid precursor of catecholamines, is obtained both from dietary intake and existing protein stores. The maintenance of catecholamine concentrations, during starving, suggests that existing stores were able to provide adequate tyrosine, and any other nutrients required, for catecholamine biosynthesis. However, if during the fasting period the fish encountered an acute stress which necessitated an elevation of plasma catecholamines, then nutrient deprivation may compromise the ability of the chromaffin cells to synthesize additional catecholamines and maintain storage levels.

A possible chronic or frequent stress that a fish may encounter is chasing by larger fish. In rainbow trout, an experimental protocol of twice daily chasing to exhaustion for a period of 5 days, markedly elevates plasma catecholamine levels [297]. Presumably, constant release of catecholamines during chasing would deplete, in part, the levels of catecholamines within the chromaffin cells. The sympathetic nervous stimulation of the chromaffin cells, in conjunction with adrenaline release and elevated plasma cortisol levels following chasing [71], would presumably stimulate the biosynthesis of catecholamines by increasing the activity of TH, $D\beta H$ and PNMT (see above). Such a chasing protocol however, [238] did not alter the levels of catecholamines stored within the kidney and posterior cardinal vein suggesting that catecholamine biosynthesis is able to regulate and maintain the normal levels of catecholamines within the chromaffin cells. The ability of trout to maintain stored catecholamines at a 'normal' level, despite repeated release due to chasing, ensures that sufficient quantities are available to allow for an elevation of plasma catecholamines should another acute stress be encountered.

The aforementioned modulating effects on catecholamine storage levels discussed above have focused on conditions imposed by the environment, including manipulation by investigators (i.e. anoxia, pollution, food deprivation and chasing). However, internal hormonal factors are also capable of influencing the levels of stored catecholamines. The effects of corticosteroids, produced by steroidogenic interrenal cells (i.e. the adrenal cortex or its homologue), on catecholamine biosynthesis in mammalian species has received substantial attention over the past decade. In particular, corticosteroids, such as cortisol, can increase levels of stored adrenaline in the mammalian adrenal gland by increasing the activity of PNMT [29,84,129,300,301]. Corticosteroids, in mammalian species, can cause an increase in PNMT activity by influencing gene transcription, messenger RNA stability, translation, and enzyme activity and stability [303]. However, few studies have investigated possible interactions between corticosteroids and catecholamine biosynthesis in fish and it appears as if, unlike in mammals, cortisol does not increase *in vitro* PNMT activity in rainbow trout [137,175].

Treatment of rainbow trout with an intraperitoneal implant of cortisol suspended in coconut oil results in a chronic elevation of plasma cortisol levels [227,279] as cortisol is slowly released, from the implant, into the body fluid over a long period of time. Cortisol treatment for 1 day had no effect on the level of stored catecholamines in the kidney and posterior cardinal vein whereas following 3 and 7 days of treatment, levels of stored noradrenaline and adrenaline were elevated over control levels [241]. This increase in stored catecholamines was not accompanied by an increase in

PNMT activity, measured *in vitro*, on either day [241]. Thus, it is likely that the effect of cortisol treatment on the enhanced level of catecholamine storage was occurring prior to the conversion of noradrenaline to adrenaline. Chronic cortisol treatment can increase the activity of D β H in rainbow trout, suggesting that cortisol may function by decreasing the degradation of this enzyme [137]. Thus, whilst cortisol in higher vertebrates appears to enhance the activity of PNMT, in lower vertebrates, including fish, cortisol may exert effects on other enzymes of the Blaschko pathway.

3. Situations eliciting catecholamine secretion

Catecholamines are mobilized into the circulation of fish during a variety of stressful situations [235] which in general either require modulation of cardiorespiratory function or mobilization of energy reserves. The magnitude of the elevation in plasma catecholamine levels can vary depending upon the type of stress imposed, the severity of the stress and the species. Various levels of plasma catecholamines in five teleost species (*O. mykiss*, *Salmo fario*, *G. morhua*, *C. carpio* and *Platichthys stellatus*) in response to various stressors (external hypoxia, hypercapnia, exhaustive and violent exercise, air exposure, acid infusion or anaemia) are reviewed in reference [267]. Additionally, anaesthesia during experimental manipulation also produces a transient elevation of plasma catecholamine levels [126]. It is evident from the studies compiled in the aforementioned review that there are marked differences in the levels of plasma catecholamines observed in different species in response to different types of stress. The dominance of either adrenaline or noradrenaline in the circulation during a stressful period is not only dependent upon storage levels [25,28,41,42,66,220] but also on the rate of metabolic degradation [56,191,192], accumulation of catecholamines into tissues [40,190,191,271,272] and the 'ability' of chromaffin cells to secrete catecholamines [239].

In mammals, plasma catecholamines can originate not only from the adrenal chromaffin cells but also from sympathetic neurons. However, in fish (Atlantic cod, [219] overflow from peripheral adrenergic nerve terminals does not significantly contribute to the elevation of plasma catecholamine levels.

3.1. Catecholamine release during hypoxia

One of the more prevalent environmental/experimental conditions eliciting an elevation of plasma catecholamine levels is external hypoxia [14,28,36,41,89,92,138,140,177,219,225,226,231,235,239,246,268]. Additionally, plasma catecholamine levels are also elevated during air exposure [94,203]. The release of cate-

cholamines into the circulation during hypoxia can be influenced by the severity of the hypoxic exposure. In the Atlantic cod, a gradual decrease in water P_{O_2} caused an elevation of plasma adrenaline levels whereas a rapid decrease in water P_{O_2} caused a simultaneous elevation of both adrenaline and noradrenaline [219]. Also in the cod, during the imposition of a hypoxic stress, noradrenaline appears in the blood prior to adrenaline [91].

Depending upon the severity of environmental hypoxia, either the partial pressure of oxygen ($P_{a_{O_2}}$) in the arterial blood or both the $P_{a_{O_2}}$ and the arterial oxygen content may decrease. Given the sigmoidal nature of the oxygen equilibrium curve and the obligate relationship between $P_{a_{O_2}}$ and oxygen content [287,288], it has proven difficult to distinguish between the effects of these two variables on promoting catecholamine release during hypoxia. A linkage between blood oxygen content/haemoglobin- O_2 saturation and plasma catecholamine levels has been suggested by several studies. First, anemic fish release catecholamines into the circulation [125,222] even under hyperoxic conditions [222]. In anemic fish, haemoglobin- O_2 saturation is not lowered, suggesting a specific role in the lowering of blood oxygen content in causing release. Second, catecholamine release during hypercapnic acidosis in trout is a result of the associated hypoxemia (owing to the Root effect) rather than the acidosis itself ([222]; see below). Third, one study [89] reported that the $P_{a_{O_2}}$ threshold for release was substantially lowered after repeated episodes of acute hypoxia suggesting that haemoglobin- O_2 affinity was raised after the initial episode of hypoxia (a result, at least in part, of catecholamine release [193] and thus led to the lowering of the $P_{a_{O_2}}$ threshold).

In order to elucidate the proximate stimulus (a depression of $P_{a_{O_2}}$ or arterial oxygen content) for the release of catecholamines during hypoxia, rainbow trout and American eels were exposed to varying degrees of acute (30 min) hypoxia (90–35 torr water P_{O_2}) [225]. Based on *in vivo* oxygen equilibrium curves, the P_{50} values (the $P_{a_{O_2}}$ at which the oxygen content of the blood is 50% of its maximal value) in the rainbow trout, and American eel were 23 and 11 torr, respectively. Thus the affinity of the oxygen-haemoglobin binding reaction is substantially enhanced in the eel over the trout. During hypoxic exposure, defined $P_{a_{O_2}}$ and oxygen content thresholds for the release of catecholamines were determined. While the $P_{a_{O_2}}$ thresholds differed in the two species, in accordance with the different haemoglobin- O_2 binding affinities, the oxygen content thresholds were almost identical, corresponding to approximately 45–60% haemoglobin- O_2 saturation. The similar oxygen content thresholds for release imply that a depression of blood oxygen content, rather than $P_{a_{O_2}}$, is the proximate stimulus for catecholamine release during hypoxia.

However, the aforementioned study [225] could not exclude the possibility that a depression of P_{aO_2} is the proximate stimulus for release with trout and eel possessing intrinsically different P_{aO_2} thresholds. In order to differentiate between these two possibilities, rainbow trout were acclimated [226] to either 5 or 15°C for a period of 2 months in order to manipulate the intrinsic properties of haemoglobin-oxygen binding and to assess the impact on the dynamics of catecholamine release during hypoxia. Temperature acclimation led to an enhanced haemoglobin- O_2 binding affinity at the lower temperature with P_{50} values of 14 and 27 torr in the 5 and 15°C fish, respectively. During exposure to acute hypoxia, the P_{aO_2} threshold at which catecholamines were released into the circulation was lower in the 5°C fish (24 torr) than in the 15°C fish (35 torr). However, in agreement with reference [225], release thresholds calculated on the basis of arterial blood oxygen content were similar at both temperatures. Thus, the results of these aforementioned studies [89,125,222,225,226] provide compelling evidence that a depression of blood oxygen content is the modality which triggers the mobilization of catecholamines into the circulation rather than a depression of P_{aO_2} .

3.2. Catecholamine release during hypercapnia/acidosis

One of the primary roles of circulating catecholamines in fish is to maintain or elevate blood oxygen levels under conditions that depress blood oxygen content. The hydration of CO_2 into protons and bicarbonate ions ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$) often results in a respiratory acidosis accompanying hypercapnic exposure (an increase in CO_2 within the water). Given the detrimental effects of H^+ and CO_2 on haemoglobin-oxygen binding, it is not surprising that plasma catecholamine levels are often elevated during exposure to environmental hypercapnia [123,221,222, 224]. Additionally, the introduction of acid into the water [304] and conditions of metabolic acidosis [14,35] are also associated with elevated plasma catecholamine concentrations.

Various studies have reported a correlation/relationship between blood pH and plasma catecholamine levels [35,225,263]. However, given the relationship between blood oxygen content and acid-base status in teleost fish, particularly rainbow trout [221,231,266,267] and the promotion of hypoxemia during acidosis via Root and Bohr effects, it is difficult to determine if a decrease in blood pH is sufficient, in the absence of hypoxemia, to cause the secretion of catecholamines. However, several studies [14,222] demonstrated that acidosis does not promote catecholamine secretion in the absence of blood hypoxemia suggesting no direct role for acidosis in catecholamine secretion.

An additional complicating factor in assessing the role of acidic conditions in promoting catecholamine release is the difficulty in distinguishing the cause of release from the consequences of catecholamine release. In teleosts, circulating catecholamines can activate a Na^+/H^+ antiporter on the red blood cell that extrudes protons from the red cell in exchange for plasma sodium ions causing acidification of the plasma and alkalization of the erythrocyte [184,194,195,267]. The subsequent increase in red blood cell intracellular pH can promote an increase in blood oxygen content through Bohr and Root effects [85,120,193,195,231, 247]. Given red blood cell Na^+/H^+ exchange, an elevation of plasma catecholamines is often accompanied by a decrease in plasma pH and therefore it is difficult to separate the cause of catecholamine secretion from the effects of release. It would appear that during exposure to hypercapnic conditions, the mobilization of catecholamines into the circulation is a result of a lowering of blood oxygen content due to Bohr and Root effects rather than a direct effect of pH per se.

3.3. Catecholamine release during exercise

Fish exposed to a variety of regimens of exhaustive or violent exercise release catecholamines into the circulation in varying quantities [18,43,44,101,169,178,188, 208,210,228,234,245,263,274,292,294–297]. During exercise, catecholamines will exert energy mobilizing effects and thus increase the blood borne energy supplies via activation of liver glycogenolysis and gluconeogenesis along with an inhibition of glycolysis [180–182,214,215]. Additionally, the catecholamine-induced enhancement of gas transfer across the gills, and oxygen transport in the blood, will prove beneficial during exercise.

Contrary to the catecholamine-mobilising effect of exercise in many fish species, this form of perturbation does not cause an elevation of circulating catecholamine levels in notothenioids (i.e. two nototheniids, *N. coriiceps*, *N. rossii* and an icefish, *C. aceratus*) [77]. Additionally, handling stress also fails to initiate the mobilisation of catecholamines in these fish [67,76]. The authors of these studies [67,77] suggest that the low levels of circulating catecholamines may reflect a greater propensity of the autonomic cholinergic system, rather than blood-borne catecholamines, to regulate certain aspects of the stress response in these species.

3.4. The effects of softwater on catecholamine release

Diminished resting levels of circulating catecholamines were observed in rainbow trout following three weeks of acclimation to ion-poor water (softwater [229]). Exposure of freshwater rainbow trout

[16,110,111,155–157,223,230,256] and other teleosts [164] to softwater induces a proliferation of gill chloride cells which thicken and cover the respiratory (pavement) cells. The resulting thickening of the blood to water diffusion distance can subsequently impair gas transfer leading to a decreased arterial P_{aO_2} and an increased P_{aCO_2} [110]. Given the beneficial effects of catecholamines on both gas transport across the gills and transfer within the blood [231,235,267], it is likely that circulating catecholamine levels are elevated during the early stages of softwater acclimation in order to compensate for the detrimental effects of a thickening in the blood to water diffusion distance. Given the chronic imposition of this stress (softwater exposure), it is likely that a continual release of catecholamines may occur which ultimately results in a depletion of storage levels and/or desensitization of the release process. Both of these factors could lead to an attenuation of plasma catecholamine levels following the 3 weeks of acclimation to softwater conditions.

The dynamics of catecholamine release during exposure to acute hypoxia can also be modified by softwater acclimation. Upon exposure to graded levels of environmental hypoxia, softwater-acclimated rainbow trout mobilize catecholamines into their circulation at a significantly higher P_{wO_2} than trout maintained under normal ionic conditions [229]. Under softwater and control conditions, defined arterial P_{O_2} thresholds for catecholamine release were 24 and 40 torr in control and softwater-acclimated trout, respectively. The elevated release threshold in the softwater acclimated fish arises from the downward displacement of the P_{aO_2} – P_{wO_2} relationship in combination with a left shifted oxygen equilibrium curve. The elevated release threshold in softwater acclimated fish affords the fish with the physiological benefits of an elevation of plasma catecholamine levels [231] at a time when gas transfer/transport may be compromised by the increased diffusion distance.

In addition to reduced resting plasma catecholamine levels and an increased P_{aO_2} threshold for the release of catecholamines during hypoxia, the absolute magnitude of plasma catecholamine levels, during severe hypoxia (P_{wO_2} = 30–35 torr), was diminished in softwater acclimated rainbow trout [229]. As mentioned above, either a depletion of storage levels or desensitization of the release process may lead to diminished plasma levels during stress, including hypoxia (see also modulation of cholinergic-induced catecholamine release below). Alternately, depleted plasma levels may also reflect changes in the rates of catecholamines synthesis and/or degradation.

4. Mechanisms and modulation of catecholamine release

4.1. Cholinergic control of catecholamine release

4.1.1. Chromaffin cell cholinergic pharmacology

Undoubtedly the primary mechanism leading to the secretion of catecholamines from the chromaffin cells in teleost and elasmobranch fish is stimulation of these cells by the sympathetic nervous system [2,116,199,201,202,219]. More precisely, chromaffin cells are innervated by pre-ganglionic sympathetic fibers which release the neurotransmitter acetylcholine (ACh) (and possibly others; [112,237,284]) onto the chromaffin cells where it interacts, with cholinergic receptors (cholinoceptors), to initiate the secretion of catecholamines via exocytosis ([37]; Fig. 2). In the majority of vertebrate species studied to date, including fish, it appears as if the nicotinic receptor is the predominant cholinergic receptor present on chromaffin cells (see below).

Stimulation of nicotinic cholinoceptors ultimately leads to the opening of calcium channels and the entry of Ca^{2+} ions into the cell from the extracellular fluid ([37–39,99,270]; see Fig. 1). The ensuing elevation of intracellular calcium levels triggers a series of events, including rearrangement of the cytoskeleton, which allows the catecholamine-containing secretory granules to move towards and fuse with the plasma membrane thereby releasing their contents into the extracellular space via exocytosis (Fig. 2). The process of exocytosis involves multiple stages and substances which mediate the movement of secretory vesicles to the plasma membrane, the docking of the vesicles to the plasma membrane, fusion of granule and plasma membranes to allow release of the contents and the ultimate recovery of the granule membrane via endocytosis [37].

The nicotinic nature of the cholinergic-induced catecholamine secretory response from fish chromaffin cells has been demonstrated in several studies. In the Atlantic cod, *G. morhua*, either electrical stimulation of the sympathetic nerves which innervate the head kidney, or application of acetylcholine to the head kidney, caused catecholamine release from in situ perfused preparations [2,203,219,282]. The secretion of catecholamines in response to either of these stimuli can be prevented by treatment of the preparation with the ganglionic blocker, hexamethonium, that inhibits nicotinic cholinoceptors.

Although stimulation of the nicotinic cholinergic receptor, and the ensuing signal transduction events following receptor activation, is the primary mediator of cholinergic-induced catecholamine secretion, the involvement of muscarinic cholinoceptors in this process is also common across various vertebrate phyla (Fig. 2). Indeed in non-fish vertebrate species, stimulation of chromaffin cells with muscarinic agonists can enhance nicotinic-

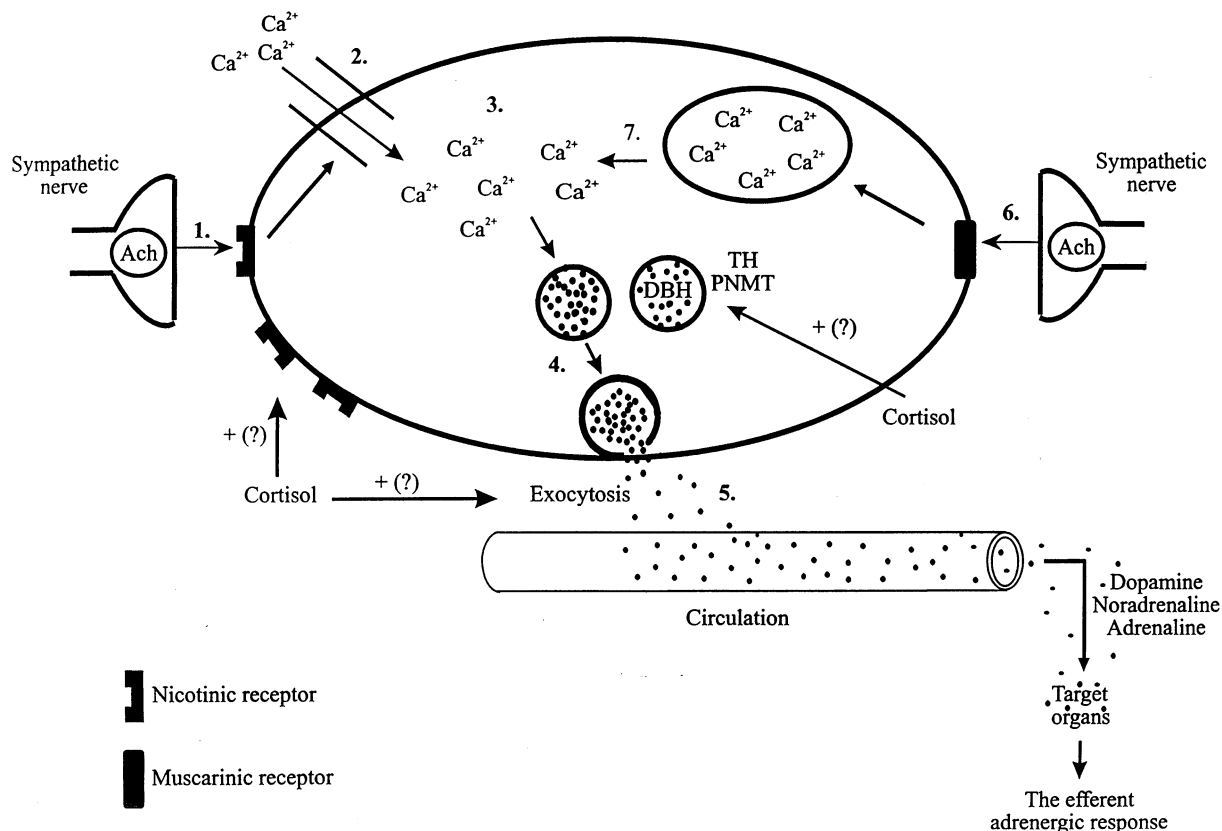


Fig. 2. A schematic diagram of the cholinergic pathway of catecholamine secretion from fish chromaffin cells. Acetylcholine released from sympathetic nerve fibers will stimulate the chromaffin cells by interacting with nicotinic [1] or possibly muscarinic [6] cholinergic receptors. Stimulation of the nicotinic receptor ultimately leads to the opening of a calcium channel and the entry into the cell of Ca^{2+} ions [2]. Muscarinic receptor activation leads to the release of Ca^{2+} from intracellular stores [7] such as the endoplasmic reticulum. The involvement of these two receptor types in the cholinergic control of catecholamine secretion can vary amongst fish species (see text). The ensuing rise of intracellular Ca^{2+} levels [3] can lead to a series of intracellular events, including rearrangement of the cytoskeleton [37], facilitating the movement of secretory granules to the plasma membrane [4] where they release their contents via exocytosis [5]. The catecholamine hormones can then diffuse into the blood where they circulate until reaching the appropriate target organs and initiate the efferent limb of the adrenergic response. The corticosteroid cortisol can enhance catecholamine storage levels possibly by up-regulating enzymes within the biosynthetic pathway (see text). Also, cortisol may influence the number/affinity of cholinergic receptors on the chromaffin cell membrane, sensitising the release process to sympathetic nerve stimulation.

evoked release [90], inhibit nicotinic-evoked release [49,70,262], stimulate secretion in the absence of nicotinic agonists [21,143,159,269] or exert no effect on catecholamine secretion [166]. Thus, amongst non-fish vertebrate species so far studied, cholinergic-induced secretion of catecholamines from chromaffin cells is certainly not confined to a series of nicotinic receptor-evoked events.

As with other vertebrate species, it would appear as if the presence of muscarinic receptors on chromaffin cells in fish might also exhibit substantial variability. In rainbow trout, *O. mykiss*, the release of catecholamines in situ can be stimulated by treatment with the mixed nicotinic/muscarinic agonist carbachol (carbamylcholine; [93,238–241]) an analogue of the endogenous neurotransmitter acetylcholine which exhibits enhanced resistance, compared with acetylcholine, to degradation by endogenous cholinesterases. Treatment of the rainbow trout perfusion preparation with hexamethonium

inhibited the release of noradrenaline and attenuated the release of adrenaline in response to carbachol [93], suggesting a role for the muscarinic receptor in the cholinergic release of catecholamines in this species. The incomplete inhibition of carbachol-evoked adrenaline release with hexamethonium implies a role for muscarinic receptors in the secretion of adrenaline, but not noradrenaline, suggesting a possible differential distribution of muscarinic cholinergic receptors on the different types of chromaffin cells (adrenaline- and noradrenaline-containing cells, see above).

In another teleost species, the American eel (*A. rostrata*), the muscarinic cholinergic receptor does not appear to mediate the secretion of catecholamines from chromaffin cells. Administration of carbachol to an in situ perfused posterior cardinal vein preparation in this species also stimulated both adrenaline and noradrenaline secretion which was abolished with hexamethonium (Fig. 1G) yet unaffected by the classical

antimuscarinic, atropine [240]. Additionally, catecholamine secretion in this species was also evoked, in situ, by application of the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) but not by the muscarinic agonist, pilocarpine. Secretion in response to DMPP was, like carbachol-evoked release, abolished by pre-treatment with hexamethonium. Given these results [93,240] it appears as if a muscarinic receptor is involved in catecholamine, particularly adrenaline, secretion in rainbow trout, but not in American eel. Therefore, as with non-fish vertebrates, the type of cholinergic receptor mediating the cholinergic secretion of catecholamines in fish may also exhibit species to species variability.

Although stimulation of chromaffin cells by the sympathetic nervous system is undoubtedly the primary stimulus evoking the secretion of catecholamines from chromaffin cells in most fish species, it is clearly not the exclusive pathway of release. The presence of other mechanisms leading to catecholamine secretion in teleost fish is evident from experiments in which the sympathetic fibers innervating the chromaffin cells are sectioned in order to eliminate sympathetic stimulation of these cells. Bilateral sectioning of spinal nerves 1–4 innervating the head kidney in the Atlantic cod attenuated but did not abolish the elevation of plasma catecholamines during air exposure [283], after exhaustive exercise [44] or during environmental hypoxia [219]. It is apparent therefore, that mechanisms other than cholinergic pathways are involved in the process of catecholamine secretion in this species. Non-cholinergic control of release is discussed in the final section of this review.

4.1.2. Differential secretion of catecholamines in response to cholinergic stimulation

In addition to differences in the nature of cholinergic pharmacology on the chromaffin cells of rainbow trout and American eels, there are also apparent differences in the nature of the secretory response in these two species with respect to the quantity of catecholamines released. Exposure of rainbow trout and American eels to severe environmental hypoxia ($P_{wO_2} = 30\text{--}35$ torr; [225,226]) results in a substantial elevation of plasma catecholamines in the rainbow trout (100–300 nmol l⁻¹) yet very moderate increases in the eel (2–5 nmol l⁻¹). These widely different plasma catecholamine levels cannot be ascribed to the differences in oxygen-haemoglobin binding and release thresholds (see above). Additionally the 4-fold difference in levels of stored catecholamines in these two species (see above) cannot account for the approximately 100-fold difference in the plasma values observed during severe hypoxia.

In an attempt to elucidate the mechanism(s) underlying these differences in plasma catecholamine levels, the

‘ability’ (i.e. efficacy/efficiency) of the chromaffin cells in these two species to release catecholamines, in situ, in response to stimulation with carbachol was examined [239]. In response to this stimulus, rainbow trout chromaffin cells secreted substantially greater quantities of catecholamines than eel chromaffin cells. The affinity of the secretory process, for carbachol, was equivalent in both species suggesting that chromaffin cells in rainbow trout possess greater numbers of cholinergic receptors than those in the eel or that one or more post-receptor events is amplified in the trout compared to the eel. Also, these differences may be explained if there are a greater number of chromaffin cells in the trout, however, to date this has not been demonstrated. Additionally, it is possible that eel chromaffin cells are less densely innervated by cholinergic fibers than those in the trout, although this would account for the in vivo but not the in situ differences. This differential ability to respond to cholinergic stimulation may explain the interspecific differences observed in plasma catecholamine levels during exposure to environmental hypoxia.

Unlike teleost fish, the chromaffin cells in cyclostomes/agnathans (i.e. hagfish and lampreys) lack any form of extrinsic innervation [15,127,128]. As such, the primary mechanism of catecholamine secretion seen in other vertebrates (i.e. neuronal stimulation by the sympathetic nervous system) is lacking. Interestingly however, the cholinergic agonist carbachol is capable of eliciting catecholamine release, in situ, in the Atlantic hagfish, *M. glutinosa* [220]. However, it is unlikely that stimulation of the chromaffin cells by endogenous acetylcholine is a physiologically relevant stimulation given that this neurotransmitter does not circulate in the blood. It is possible that the presence of cholinergic receptors on the chromaffin cells in this species represents a fundamental property of these cells reflecting their embryological origin (modified post-ganglionic cells derived from neural crest) and is independent of extrinsic innervation. Alternately, a population of chromaffin cells in this species (i.e. those in the great veins) might be innervated, and therefore possess cholinergic receptors, while others (i.e. in the hearts, which were the focus of earlier morphological studies) are not [220]. Potential physiological mechanisms causing the in vivo mobilization of catecholamines in this species are discussed in the following section (non-cholinergic control).

4.1.3. Modulation of cholinergic control of catecholamine release

Just as storage levels of catecholamines can be modulated by factors such as anoxia, pollution, nutritive stress, physical stress and hormones, the process of cholinergic-induced catecholamine secretion can also be influenced by the prior history of the animal including

the aforementioned conditions. Stresses such as physical disturbance (e.g. handling or chasing) [23,188,245,280] and starvation/fasting [23,154,158,168,259,261,280] are encountered frequently in aquaculture and studies on fish physiology and metabolism. Similar stresses are also undoubtedly encountered in the natural environment.

Chasing rainbow trout to exhaustion once a day for either 1, 3 or 7 days caused an immediate increase in plasma catecholamine levels that remain elevated for up to 30 min post-exhaustion [228]. However, on the 3rd and 7th days of chasing, plasma catecholamine levels immediately post-chasing were significantly depressed compared with the post-chasing levels seen after only a single day of chasing. Given that a similar chasing protocol did not result in a depletion of catecholamine stores in this species [238], the depression of plasma levels on the 7th day suggests an inhibition/desensitization of catecholamine release following repeated stress. This attenuation of release is consistent with the desensitization of carbachol-evoked catecholamine release, *in situ*, following 5 days of twice daily chasing to exhaustion [238]. Given that the sensitivity of release, *in situ*, was diminished rather than a decrease in the maximal quantity released, it is likely that the attenuation of *in situ* release resulted from a decrease in the affinity of cholinergic receptors for carbachol rather than a reduction in receptor numbers. The reduction in plasma catecholamine levels following repeated stress, *in vivo*, likely functions to prevent an over expression of the efferent adrenergic stress response in accordance with general theories regarding receptor desensitization/down-regulation [57,102,118].

While physical stress (chasing) appears to desensitize catecholamine release both *in vivo* and *in situ*, nutritive stress (fasting for 2 months) attenuates the levels of stored catecholamines (see above) but does impair the ability of the chromaffin cells to secrete catecholamines, *in situ*, in response to carbachol [238]. During long-term food deprivation, fish become hypometabolic [183] and therefore it is conceivable that under such circumstances fish would desensitize the process of catecholamine release in order to minimize any catecholamine-induced glycogenolysis. However, a 2-month regimen of fasting did not produce such desensitization and indeed perhaps a more appropriate response would be to down-regulate the efferent adrenergic response on hepatocytes and muscles rather than attenuating the mobilization of catecholamines. In this manner, during an acute stress, the beneficial effects of catecholamines on gas transfer/transport can be maintained while avoiding undesirable metabolic effects.

The secretion of catecholamines into the circulation is generally considered to be a component of the acute stress response. However, the overall stress response in vertebrates also consists of a chronic component that

involves the release, into the circulation, of corticosteroids [71,101,176,233,280]. Cortisol, the major corticosteroid produced by the steroidogenic interrenal cells in fish (which like the chromaffin cells are located within the head kidney in teleost fish, [189]), is released into the circulation upon stimulation of the interrenal cells by adrenocorticotropic hormone (ACTH) released from the pituitary gland [17]. In rainbow trout, 7 days of chronic cortisol treatment enhanced the sensitivity of *in situ* catecholamine release in response to stimulation with carbachol without altering maximal levels of release [241]. It is possible that the enhanced sensitivity of the release process following cortisol treatment results from a cortisol-mediated increase in the affinity of cholinergic receptors for carbachol. Alternately, cortisol may induce the *de novo* synthesis of cholinergic receptors already present on the chromaffin cell membrane. Additionally, any post-receptor modifications within the signal transduction pathway could account for the enhanced ability to release catecholamines. In addition to enhancing cholinergic-induced release of catecholamines, cortisol treatment also increased *in situ* basal adrenaline, but not noradrenaline, secretion, an observation which may reflect the enhanced levels of stored catecholamines following cortisol treatment (see above; Fig. 2).

Clearly cholinergic control of catecholamine release is not a static process but is subject to modification and modulation by a variety of factors, intrinsic and extrinsic. Although the cholinergic pathway of release is the primary mechanism inducing catecholamine release, the presence of non-cholinergic mechanisms creates the potential for a dynamic and diverse secretory response from fish chromaffin cells.

4.2. Non-cholinergic control of catecholamine release

Recent advances in the assessment of the role of catecholamines in physiological control have described multiple patterns in the activation of catecholamine release that vary with the nature and severity of stress [149]. In addition to cholinergic innervation, there is considerable evidence that a large number of non-cholinergic neurotransmitters and hormones can also evoke or modulate adrenaline and noradrenaline release in vertebrates [37,167]. Overall, the control of chromaffin cell activity in mammals involves neural signals from cholinergic and peptidergic nerve fibers, and humoral signals of circulatory, paracrine, or autocrine origin. In fish, a number of recent studies suggest that the control of catecholamine release may also involve all of the above mechanisms and that significant species differences may also exist ([25–27,83,93,142,237,241]; Fig. 3). In agnathans (hagfish and lamprey) however, the chromaffin cells are non-innervated ([25,66]) and

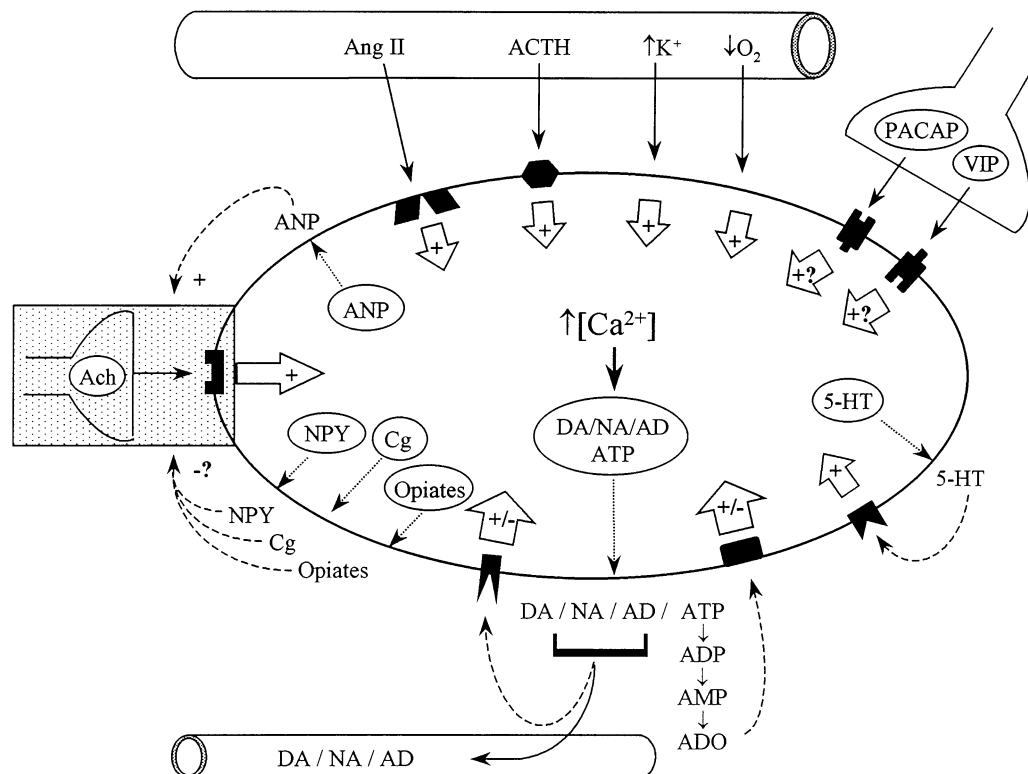


Fig. 3. Schematic diagram of the potential non-cholinergic mediators of catecholamine secretion in a generic fish chromaffin cell. Circulating angiotensin (II Ang II) and adrenocorticotropic hormone (ACTH) can increase (+) catecholamine secretion by stimulating their respective receptors on the surface of chromaffin cells. Similarly, an increase in K^+ or a decrease in oxygen can directly increase catecholamine secretion. The neurotransmitters pituitary adenylyl cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) have been identified in chromaffin cells associated-nerve fibers in several fish species, and based on their effects on catecholamine secretion in mammals [54,112], these neurotransmitters may have stimulatory (+?) effects on catecholamine secretion in fish chromaffin cells. The secretion of serotonin (5-HT) by chromaffin cells, or other cell types in the head kidney, may lead to an increase in catecholamine secretion. Adenosine (ADO), presumably the product of ATP breakdown following exocytosis of catecholamines, can either increase or decrease (+/-) catecholamine secretion. In addition to being released into the circulation, dopamine (DA), noradrenaline (NA), and adrenaline (AD), may either increase or decrease catecholamine secretion. There is immunohistochemical evidence for the presence of opiates, chromogranins (Cg), and neuropeptide Y (NPY), in the chromaffin cells of some fish species. Based on their effects on catecholamine secretion in mammals [50], the above peptides may decrease (-?) the secretion of catecholamines elicited by cholinergic stimulation. Atrial natriuretic peptide (ANP) released by chromaffin cells may increase the acetylcholine-stimulated (ACh) catecholamine secretion. Although the secondary messenger signals activated by these various secretagogues are not known, their effects on catecholamine secretion are most likely mediated by changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]$) [99]. Solid arrows represent endocrine or neuronal pathways, dashed arrows represent paracrine or autocrine pathways, and dotted arrows represent exocytosis of secretory granules.

thus the control of catecholamine release may be entirely via non-cholinergic mechanisms. Although several potential non-cholinergic secretagogues of catecholamines have been identified in fish, their relative contribution to the control of catecholamine release in vivo, and the physiological conditions under which they act have yet to be characterized.

4.2.1. Non-humoral agents

4.2.1.1. Potassium. Although perfusion of the chromaffin tissue with supraphysiological doses of K^+ results in a non-specific depolarization of the chromaffin cell and a marked release of catecholamines in all vertebrate species examined [37,220,239], the role of physiological concentrations of K^+ in the control of

catecholamine release appears to be species specific. Whereas a moderate increase in perfusion saline $[K^+]$ in an in situ preparation of *M. glutinosa* does not affect catecholamine release [220], injections of physiological doses of potassium ions does cause a dose-related increase in the concentration of circulating catecholamines in the dogfish shark, *S. acanthias* [206]. In view of the latter results, it was suggested [208] that the increase in plasma K^+ observed during and after exercise in *S. acanthias* may be, at least in part, responsible for the release of catecholamines and maintenance of plasma catecholamine levels in the post-exercise period. Furthermore, the stimulatory effects of K^+ in the dogfish are not blocked by pre-treatment with the cholinergic receptor antagonist hexamethonium [209,210], suggesting that the effects of K^+ on cate-

cholamine release are not mediated through the nicotinic receptor.

4.2.1.2. Oxygen. As mentioned above, a lowering of blood oxygen content may be one of the principle factors initiating the release of catecholamines from the chromaffin tissue in teleosts. Therefore, it is significant to note that both neuronal and non-neuronal mechanisms may be involved in mediating the control of catecholamine release during hypoxia in these fish. In the Atlantic cod (*G. morhua*), while sectioning the spinal nerves supplying the head kidney prevented a hypoxia-induced ($P_{wO_2} = 40$ torr) increase in plasma noradrenaline, it had no effect on the increase in plasma adrenaline [219]. Moreover, hypoxic blood ($P_{aO_2} = 23$ torr) directly stimulated adrenaline secretion in an in situ head kidney perfusion preparation of *G. morhua* [219].

Studies using cultured bovine chromaffin cells (BCC) have also shown that basal catecholamine release is elevated by anoxic incubation conditions [124]. However, while catecholamine release evoked by supraphysiological doses of K^+ in BCC may be enhanced after 10 min of anoxic incubation [124], several studies have also shown that hypoxia inhibits high- K^+ induced catecholamine release [124,161,162]. Moreover, anoxic incubation of BCC inhibits catecholamine release elicited by nicotine [161] and decreases the affinity of the nicotinic cholinergic receptors [163].

4.2.1.3. Acidosis. While hypoxemia appears to be an essential requirement for catecholamine release following exposure to hypercapnic and/or acidotic conditions in vivo, the effect of regional acidosis on catecholamine release from the chromaffin tissue of the head kidney has yet to be directly assessed in teleosts. In agnathans, despite the observation that prolonged and severe hypoxic exposure can result in both blood acidosis and catecholamine release [27,28,220], lowering the pH (from 8.1 to 7.0) of the saline perfusing the principle chromaffin tissue of *M. glutinosa* did not elicit catecholamine secretion [220]. Additionally, although exposure of cannulated *Petromyzon marinus* to CO_2 is associated with a significant increase in plasma catecholamines [66], it remains to be seen whether this is due to a direct effect of blood acidosis on the chromaffin tissue.

In mammals, the influence of pH on the control of catecholamine release from the adrenal medulla is controversial. In BCC for example, while noradrenaline secretion evoked by carbachol was enhanced after a decrease in pH_i (7.2–6.8 [139]), acidosis of the incubation buffer (pH 7.4–6.0) was also shown to reduce nicotine-induced noradrenaline release and the corresponding intracellular Ca^{2+} transients [148]. In addition, perfusion of isolated rat adrenal glands with an

acidified buffer solution elicited an increase in intracellular Ca^{2+} transients (detectable at pH 7.0 and increased until pH \sim 6.4) while stimulating adrenaline, but not noradrenaline, secretion [98].

4.2.2. Humoral agents

4.2.2.1. Serotonin. In addition to catecholamines, the head kidney region of several fish species also stores the biogenic amine serotonin (5-hydroxytryptamine; [237]). While these serotonin stores have been immunohistochemically localized in chromaffin cells of both the Atlantic cod and European eel, within the head kidney of the rainbow trout, serotonin appears to be stored in a cell-type other than the chromaffin cell [237]. Tissue extracts from the rainbow trout posterior cardinal vein contain large quantities of serotonin ($44.6 \mu\text{g g}^{-1}$; [93]), a storage level equivalent to that of adrenaline and noradrenaline [239]. Positive immunohistochemical labeling for antisera raised against serotonin was also observed in both the systemic and portal hearts of the Atlantic hagfish, *M. glutinosa* [237].

In rainbow trout, intra-arterial injections of serotonin ($50\text{--}250 \text{ nmol kg}^{-1}$) in vivo elicited the dose-dependent release of both catecholamines [93]. Similarly, bolus injections of serotonin (250 nmol kg^{-1}) into an in situ saline-perfused PCV preparation of rainbow trout also resulted in a significant increase in perfusate adrenaline and noradrenaline levels [93]. While the in situ serotonin-induced release of adrenaline in rainbow trout was abolished by pre-treatment with the serotonergic receptor antagonist methysergide, it did not abolish the catecholamine increases in vivo [93]. Serotonin (250 nmol kg^{-1}) also stimulated the secretion of both catecholamines in an in situ preparation of the Atlantic hagfish [25]. In this species, pre-treatment with methysergide abolished the serotonin-mediated release of both catecholamines [25].

Although serotonin is stored in the vicinity of catecholamines and can elicit catecholamine secretion in both rainbow trout and Atlantic hagfish, the physiological conditions under which these serotonin stores may participate in the control of catecholamine release have yet to be established. In situ, the inability of carbachol, a cholinergic agonist, to elicit serotonin release in either the rainbow trout [93] or Atlantic hagfish [25] indicates that, at least in these two species, the release of serotonin is not under cholinergic control. In the Atlantic hagfish, while injections of $[\text{Asn}^1\text{-Val}^5]$ -angiotensin II elicits the release of serotonin in situ [25], the significance of this observation remains to be confirmed in vivo.

A close association between serotonin stores and catecholamine stores is not a characteristic restricted to fish. In addition to catecholamines, the chromaffin cells of different anuran [68,69,153] and mammalian (see [88])

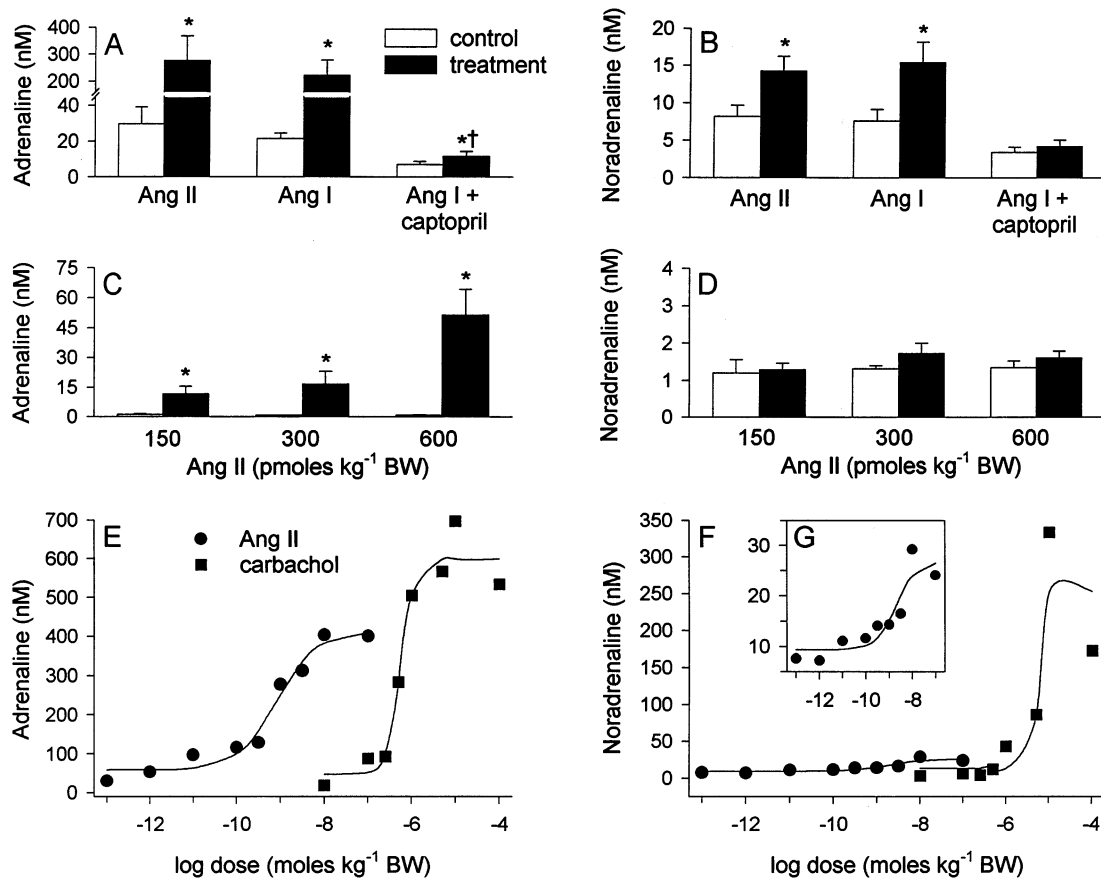


Fig. 4. (A) Perfusate [adrenaline] and (B) [noradrenaline] from in situ head-kidney preparations of *O. mykiss* given a bolus injection of either 1 nmol kg⁻¹ body weight (BW) [Asn¹-Val⁵]-angiotensin II (Ang II), [Asn¹-Val⁵-Asn⁹]-angiotensin I (Ang I), or [Asn¹-Val⁵-Asn⁹]-angiotensin I in fish pre-perfused with the angiotensin converting enzyme inhibitor captopril (10⁻⁴ mol l⁻¹; Ang I + captopril) [25]. (C) Plasma [adrenaline] and (D) [noradrenaline] from *O. mykiss* given a bolus injection of [Asn¹-Val⁵]-angiotensin II (N.J. Bernier and S.F. Perry, unpublished observations) in vivo. The open and solid bars of panels A–D represent control and maximum [catecholamine] in nM before and after the injections of angiotensins, respectively. * Significantly different from the control values for a given treatment. † Significantly different from the response obtained in the Ang I treatment. (E) Dose response curves of perfusate [adrenaline] and (F) perfusate [noradrenaline] as a function of log [Asn¹-Val⁵]-angiotensin II (solid circle; [25]) and of the cholinergic agonist carbachol (solid square; C. Montpetit and S.F. Perry, unpublished observations) in in situ head-kidney preparations of *O. mykiss*. (G) Enlargement of the dose-response curve of perfusate [noradrenaline] as a function of log [Asn¹-Val⁵]-angiotensin II.

for references) species also contain serotonin. In mammals, several studies have attributed direct peripheral catecholamine-releasing effects to serotonin [73,74,86,236]. However, although the role of the central serotonergic system in the control of the sympathoadrenal system is well documented [48], the physiological significance of the adrenal serotonergic stores in the control of catecholamine release has yet to be elucidated.

4.2.2.2. Angiotensins. There is considerable evidence that the regulatory peptides of the renin-angiotensin system (RAS), angiotensins, may contribute to the regulation of catecholamine secretion from the chromaffin tissue of the head kidney. Using a bioactive immunohistochemical probe [26], it was demonstrated that the chromaffin tissue of rainbow trout has angiotensin II binding sites which can mediate the stimulatory effects

of angiotensin II on catecholamine secretion. However, while bolus injections of the native rainbow trout angiotensin II ([Asn¹,Val⁵]-AngII) caused a dose-dependent release of both catecholamines in situ (Fig. 4 A,B), the effects of angiotensin II are more pronounced on adrenaline than on noradrenaline secretion [26]. Angiotensin I also elicits the release of both catecholamines (Fig. 4), however, most of the angiotensin I-elicited catecholamine release is indirect and requires conversion to angiotensin II [26].

Intravascular injections, in vivo, of human angiotensin II ([Asp¹-Ile⁵]-AngII) in the lumpfish, *Cyclopterus lumpus* [46] and in the dogfish, *S. acanthius* [207], and of native angiotensin II in rainbow trout (Fig. 4C,D), also elicited an increase in the circulating levels of catecholamines. In rainbow trout, angiotensin II, produced either by the systemic renin-angiotensin system or by a localized renin-angiotensin system in the

kidney [26], plays an essential role in mediating catecholamine release during hypotensive conditions (N.J. Bernier and S.F. Perry, unpublished observation). Indirect evidence also suggests that angiotensin II may elicit catecholamine release in the bowfin, *A. calva* [45], and in the American eel, *A. rostrata* [204,216]. In those species, results of adrenoceptor blockade experiments have shown that the angiotensin II-induced pressor responses are partially mediated by secondary stimulation of the sympathetic nervous system, thereby implying the possible involvement of circulating catecholamines. However, although the pressor response elicited by human angiotensin II in the Atlantic hagfish can also be abolished by adrenergic receptor blockade [46], bolus injections of rainbow trout angiotensin II had no effect on the secretion rate of noradrenaline and adrenaline in an in situ preparation from the hagfish [26].

4.2.2.3. Natriuretic peptides. Although several natriuretic peptides (NP) have been isolated and sequenced from several species of fish ([113]), and natriuretic peptides have a variety of different effects on catecholamine synthesis and release from the mammalian adrenal medulla [87,150,276], the interactions between NP and catecholamine release in fish have only been directly assessed in a few species of fish. In *C. carpio*, there is both immunohistochemical evidence for the presence of atrial natriuretic peptides (ANP) in the adrenaline-synthesizing cells of the head kidney, and autoradiographic evidence for ANP binding sites in the adrenal tissue [142]. In vitro, perfusion of *C. carpio* head kidney slices with rat ANP (10^{-7} mol l^{-1}) increases acetylcholine-evoked adrenaline release [142]. These results [142] suggest that ANP may have an autocrine and/or paracrine role in the control of catecholamine secretion in *C. carpio*. In contrast, however, despite the ability of α -adrenergic antagonists to block the hypertensive effect of ANP in the rainbow trout [205], in situ perfusion of the chromaffin tissue of trout with rat ANP (10^{-9} mol l^{-1}) or trout ventricular natriuretic peptide (VNP; 10^{-9} mol l^{-1}) does not significantly affect basal or carbachol-elicited catecholamine release (J.E. McKendry and S.F. Perry, unpublished observations).

In other vertebrate species ANP has either an inhibitory effect on catecholamine release or no effect at all. In the amphibian *Xenopus laevis*, basal or Ach-evoked noradrenaline and adrenaline release was not affected by the addition of rat ANP in vitro, and the injection of rat ANP in vivo had no effect on the circulating catecholamine levels [141]. In the rat adrenal medulla, ANP inhibits spontaneous as well as membrane depolarization- (with KCl) evoked noradrenaline release [276]. Similarly, in the perfused bovine adrenal medulla, ANP inhibits the release of catecholamines

induced by KCl-depolarizing solutions, Ach, and angiotensin II [87].

4.2.2.4. Adrenocorticotrophic hormone and cortisol. Activation of the hypothalamo-pituitary axis and release of adrenocorticotrophic hormone (ACTH) into the circulation by the pituitary is an integral part of the primary stress response of fish [22,71,260]. ACTH, in addition to stimulating corticosteroid release from the interrenal cells of the head kidney, has also been recently implicated in the control of catecholamine secretion in both the rainbow trout [241] and the Atlantic hagfish [25,220]. In rainbow trout, in situ administration of a native pituitary extract elicited the release of adrenaline, but not noradrenaline while porcine ACTH elicited a dose-dependent release of adrenaline and a smaller, non dose-dependent, release of noradrenaline. This ACTH-elicited secretion of catecholamines in the trout was unaffected by pre-treatment with the cholinergic receptor antagonist, hexamethonium, or the serotonergic receptor antagonist, methysergide, but was abolished in calcium-free media. Also, cortisol administration in situ does not evoke catecholamine release, suggesting that ACTH is acting directly on receptors on the chromaffin cell membrane to initiate catecholamine release [241]. Additionally, in rainbow trout, intra-arterial injections of porcine ACTH in vivo caused an elevation of plasma adrenaline but not noradrenaline levels [241].

In the Atlantic hagfish, in situ administration of either an extract of Atlantic cod pituitary [220] or porcine ACTH [25] elicited the release of both catecholamines which was unaffected by pre-treatment with either hexamethonium or methysergide [25]. In contrast to the effects of ACTH in the hagfish and rainbow trout, perfusion of *C. carpio* head kidney slices with ACTH stimulated cortisol secretion but did not affect the release of catecholamines [142].

In rainbow trout, although cortisol does not directly stimulate catecholamine secretion in situ, it has been demonstrated to modulate the cholinergic control of catecholamine release [241]. In trout a chronic elevation of plasma cortisol levels increased the responsiveness of the catecholamine release process in situ to low doses of the cholinergic agonist carbachol [241]. In contrast, however, the chromaffin cells of rainbow trout chased twice daily to exhaustion for 5 days, exhibited a decrease in responsiveness to carbachol in situ [238]. Therefore, while cortisol alone may sensitise the responsiveness of the catecholamine release process [241], under conditions of repeated physical stress [238] the effects of cortisol may be marked by other modulators of catecholamine release.

4.2.2.5. Opioid peptides and other bioactive peptides. The presence of opioid peptides in fish chromaffin cells was first demonstrated, using immunohistochemical tech-

niques, in the posterior cardinal vein and the head kidney of the American eel, *A. rostrata* [117]. This initial survey revealed methionine-enkephalin, leucine-enkephalin, and morphine-like immunoreactivity in the chromaffin tissue [117]. Further experiments with perfused head kidney pieces in this species revealed that, in addition to catecholamines, the chromaffin tissue secretes morphine and its precursor codeine [82]. While both of these endogenous opiates appear to modulate catecholamine secretion in the eel in vitro, the complexity of these interactions and the multiplicity of potential chromaffin cell opiate receptors does not allow for one to draw definitive conclusions regarding a possible autocrine function for these peptides [83].

Enkephalins are also present within the chromaffin cells of several amphibian (see [242] for references), and mammalian (see [75] for references) species. In addition to co-localization with catecholamines, enkephalins are also co-secreted in response to various stimuli (see [64] for references). Opioid binding sites are located on mammalian chromaffin cells, and while several studies have demonstrated that opioids can inhibit nicotinic-evoked catecholamine release from the adrenal gland [50,152], other studies have found no role for adrenal opioid peptides in modulating catecholamine secretion (see [171] for references).

In contrast to the aforementioned study [117], another study [237] did not detect any enkephalins (met-enkephalin, met-enkephalin-arg⁶-phe⁷, met-enkephalin-arg⁶-gly⁷-leu⁸) associated with the chromaffin tissue in either the rainbow trout, Atlantic cod, European eel, spiny dogfish or Atlantic hagfish. Indeed, with the exception of immunohistochemical evidence for the presence of neuropeptide Y (NPY) and peptide YY (PYY) in the European eel, the chromaffin cells of the three teleost species did not exhibit a positive labeling reaction for LPLRFamide, FMRFamide, galanin, substance P, somatostatin, or chromogranins [237]. Since several of these bioactive peptides (and others not investigated in this study [237]) are present in the chromaffin cells of different mammalian and amphibian species (see [211] and [237] for references) but absent from a number of fish species, it appears that the content of fish chromaffin cells may be less complex than those found in other vertebrates [237]. However, evidence for the presence of chromogranins in the chromaffin cells of *C. carpio* and *M. scorpius* has been reported [243,244]. Thus, in fish, as in other vertebrates, there appears to be significant species differences in the occurrence and distribution of chromaffin cell associated peptides.

4.2.2.6. Adenosine. In the adrenal medulla of mammals, ATP co-released with catecholamines from chromaffin cells during exocytosis, is degraded to adenosine within the extracellular space [72]. Through an autocrine or

paracrine pathway, adenosine, or adenosine agonists, can suppress catecholamine secretion from the adrenal medulla [51,52,151] via specific adenosine receptors on the surface of chromaffin cell [47]. Consequently, adenosine receptor blockade enhances the secretory response elicited by acetylcholine [151].

Although the presence of ATP in fish chromaffin cells, its co-release with catecholamines and breakdown to adenosine in the extracellular space remain to be investigated, there is mounting evidence that adenosine is involved in the control of catecholamine release [25,27]. In both *O. mykiss* and *E. stouti*, pre-treatment with intravascular injections of the adenosine receptor antagonist theophylline, significantly increased the concentration of circulating adrenaline following a hypoxic challenge [27]. In *M. glutinosa*, in situ perfusion of the systemic heart and PCV with the adenosine receptor agonist NECA, or the antagonist DPSPX, modified the catecholamine secretory responses elicited by ACTH, serotonin, and carbachol [25]. Overall, these experiments in *M. glutinosa* suggest that adenosine may inhibit the release of catecholamines elicited by either serotonin or carbachol, while stimulating those elicited by ACTH [25]. Both the in vivo [27] and in situ [25] results suggest that the modulatory effects of adenosine may be occurring primarily on the adrenaline-storing cells.

4.2.2.7. Histamine. Histamine can elicit a substantial secretory response from the adrenal medulla in several mammalian species [34,37]. Indeed, histamine may be the most potent non-cholinergic secretagogue of catecholamines from the isolated bovine adrenal chromaffin cell [37]. To our knowledge, the potential effect of histamine on catecholamine release has yet to be investigated in either teleosts or elasmobranchs. In the Atlantic hagfish, *M. glutinosa*, perfusion of the chromaffin tissue with histamine concentrations ranging from 0.3 to 300 μ M did not elicit catecholamine secretion [25].

4.2.2.8. Catecholamines. In the Atlantic cod, *G. morhua*, the addition of high levels of a particular catecholamine (adrenaline or noradrenaline; \sim 200 nM) into the inflowing perfusion fluid of an in situ preparation inhibited the Ach-elicited release of that particular catecholamine [219]. Thus it appears as if each circulating catecholamine in the Atlantic cod may 'auto-inhibit' its own release from the head kidney through a negative feedback control mechanism [219]. In contrast, in the lamprey, *P. marinus* [65] and the American eel, *A. rostrata* [79], there is in vivo evidence that catecholamines are catecholaminotropic (i.e. can initiate catecholamine release). While only adrenaline caused a dose-related increase of plasma dopamine and noradrenaline in the lamprey [65], each of the three catecholamines stimulated the release of the respective

other two in *A. rostrata* [79]. In the eel, these catecholaminotropic effects were unaffected by denervation of the chromaffin tissue in the head kidney [116]. In rainbow trout, continuous infusion of adrenaline does not effect the circulating levels of noradrenaline [218]. Also, while adrenaline and dopamine, but not noradrenaline, may be catecholaminotropic in the rat [80], catecholamines do not have such a property in humans [81]. As such, there appear to be complex, and species specific, interactions between circulating catecholamines and the further release of catecholamines from chromaffin tissue.

4.3. Non-cholinergic neuronal agents

In addition to acetylcholine, several non-cholinergic neurotransmitters and/or neuromodulators have been identified as potential secretagogues of catecholamines from the chromaffin tissue in vertebrates (see [167] for review). In fish, nerves fibers displaying vasoactive intestinal polypeptide (VIP) and pituitary adenylyl cyclase-activating polypeptide (PACAP) immunoreactivity have been identified in the chromaffin tissue of *G. morhua*, *O. mykiss*, *A. anguilla*, and *S. acanthias* [237]. In the axillary bodies of the elasmobranch *S. acanthias*, there are also nerve fibers exhibiting substance P-like and galanin-like immunoreactivity [237]. Although the physiological significance of these observations remains speculative, in both mammals and amphibians there is increasing evidence that VIP, PACAP, and tachykinins (the family of peptides which includes substance P) may have the ability to stimulate and/or modulate catecholamine release under physiological conditions [54,112,145,146,165,167,278,286,302,306].

5. Conclusion

This review has provided a detailed account of the afferent limb of the adrenergic stress response in fish. The control of catecholamine secretion from chromaffin cells in fish involves a multitude of neurotransmitters, hormones, chemical factors and second messenger systems. The presence of numerous cholinergic and non-cholinergic pathways of release suggests that the chromaffin cell in fish is a complex endocrine/paracrine cell with the potential for a dynamic and flexible process of secretion capable of responding to numerous environmental or physiological perturbations. Although many of the complexities of the mammalian chromaffin cell have yet to be revealed in fish, clearly there are many similarities in the afferent limb of the adrenergic stress response across the vertebrate lineage.

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