

## SEROTONIN-MEDIATED RELEASE OF CATECHOLAMINES IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

REGINA FRITSCHÉ\*, STEVE G. REID, SERGE THOMAS† and STEVE F. PERRY

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

Accepted 26 January 1993

### Summary

The effects of serotonin (5-hydroxytryptamine; 5-HT) on catecholamine release from chromaffin tissue were investigated in the rainbow trout (*Oncorhynchus mykiss*) *in vivo* and *in situ*. Intra-arterial injections of serotonin *in vivo* caused dose-dependent ( $50\text{--}250\text{nmolkg}^{-1}$ ) increases in both plasma noradrenaline and adrenaline levels. Pre-treatment of fish with the serotonergic receptor antagonist methysergide did not abolish these increases.

An *in situ* saline-perfused head kidney preparation was developed and validated to study the potential direct effect of serotonin on catecholamine release. The chromaffin cells in the preparation showed a dose-dependent release of catecholamines in response to bolus injections of the cholinergic receptor agonist carbachol ( $10^{-7}\text{--}10^{-4}\text{molkg}^{-1}$ ). The carbachol-induced release of noradrenaline, but not of adrenaline, was reduced significantly when the nicotinic receptor antagonist hexamethonium ( $10^{-4}\text{mol l}^{-1}$ ) was present in the perfusion fluid. The removal of calcium from the perfusion fluid prevented the usual release of catecholamines evoked by carbachol.

Bolus injections of serotonin ( $250\text{nmolkg}^{-1}$ ) into the inflowing perfusion fluid resulted in significantly increased levels of adrenaline and noradrenaline in the outflowing perfusate. Addition of hexamethonium to the perfusion fluid did not abolish this serotonin-induced release of catecholamines. The serotonin-induced release of adrenaline, however, was abolished totally by the addition of methysergide. Serotonin is present in high concentrations ( $44.61\pm 5.96\mu\text{g g}^{-1}$  tissue) in the anterior region of the posterior cardinal vein within the head kidney. Carbachol ( $10^{-5}\text{molkg}^{-1}$ ) did not elicit release of the stored serotonin from the perfused head kidney preparation.

We conclude that the chromaffin cells in the perfused trout head kidney preparation display characteristics similar to those of other vertebrates and that this preparation is a useful tool for studying the control of catecholamine release in fish. The results demonstrate that serotonin has a direct impact on the chromaffin cells by interacting with methysergide-sensitive receptors to initiate the release of adrenaline. The potential physiological role of serotonin on catecholamine release in trout is discussed.

\*Present address: Department of Zoophysiology, University of Göteborg2, Medicinaregatan 18, 413 90 Göteborg, Sweden.

†Present address: CNRS, URA 648, Laboratoire de Physiologie Animale, Faculté des Sciences et Techniques, Université de Bretagne Occidentale, 6 Avenue Victor Le Gorgeu, F-29287 Brest, France.

Key words: chromaffin cells, catecholamines, serotonin, *Oncorhynchus mykiss*, rainbow trout.

### Introduction

The catecholamine-containing chromaffin cells of teleost fish are located within the kidney, particularly in the anterior region or head kidney, and the walls of the posterior cardinal vein(s) which pass through the kidney. These cells are innervated by sympathetic, pre-ganglionic fibres which, upon stimulation, release acetylcholine (Jönsson *et al.* 1983; Nilsson, 1983). Acetylcholine interacts with cholinergic receptors on the chromaffin cell membranes to initiate a cascade of events that culminates in exocytosis of the contents of storage granules and, hence, the release of catecholamines (Burgoyne, 1991; Nilsson, 1983). The catecholamines stored in the chromaffin cells of fish are adrenaline and noradrenaline (Nilsson, 1983) and, to a lesser extent, dopamine (Hathaway *et al.* 1989). In addition, the indolamine serotonin (5-hydroxytryptamine; 5-HT) has been localized in frog adrenochromaffin cells (Delarue *et al.* 1992) and in the adrenal medulla of mammals (Verhofstad and Jönsson, 1983; Holzwarth and Brownfield, 1985). Immunohistochemical studies reveal the co-existence of serotonin and adrenaline in chromaffin cells of the frog inter-renal gland (Delarue *et al.* 1992) as well as in the adrenal medulla of mammals (Franzoni *et al.* 1987). Furthermore, splanchnectomized frogs showed a twofold increase in the serotonin concentration in the inter-renal gland, suggesting that serotonin and adrenaline are released together upon cholinergic stimulation (Delarue *et al.* 1992).

Stimulation of serotonergic receptors, *in vivo*, causes elevated plasma catecholamine levels in rats (Chaouloff *et al.* 1991, 1992). Recent experiments on rainbow trout demonstrated increased plasma levels of both adrenaline and noradrenaline after intra-arterial injections of serotonin (Fritsché *et al.* 1992). Simultaneously, serotonin caused branchial vasoconstriction, impairment of gas transfer, and thus blood hypoxaemia, which in itself is a stimulus for catecholamine release in fish (Perry *et al.* 1991). Other known stimuli for catecholamine release from chromaffin cells include (i) direct depolarization of the chromaffin cell membrane by potassium ions (Opdyke *et al.* 1983*a,b*), (ii) various agonists of cholinergic receptors and (iii) stimulation of the sympathetic nerves innervating the chromaffin cells. In all cases a requirement for catecholamine release is the presence of Ca<sup>2+</sup> (Nilsson, 1983; Burgoyne, 1991). Thus, serotonin could exert its stimulatory effect on catecholamine release (Fritsché *et al.* 1992) either directly by acting with serotonergic (or other) receptors on the chromaffin cells or, indirectly, by causing internal hypoxaemia.

In the present study, we have first characterized the release of catecholamines from an *in situ*-perfused head kidney preparation and then, by using both *in vivo* and *in situ* techniques, assessed several potential mechanisms underlying the serotonin-mediated release of catecholamines.

### Materials and methods

#### *Experimental animals*

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of either sex weighing between 250 and 350g were obtained from Linwood Acres Trout Farms (Campbellcroft, Ontario). Fish were held indoors in large fibreglass tanks supplied with flowing, dechlorinated city-

of-Ottawa tapwater. The temperature of the holding and experimental tanks was 12°C; the photoperiod was kept at 12h light:12h dark. Fish were fed daily with a commercial salmonid diet (Martin Feed Mills Inc.) but were not fed for 48h prior to experimentation. The experiments were performed during April and May.

#### *In vivo experiments*

Fish were anaesthetized in water containing 1:10000 (w/v) MS 222 (ethyl-*m*-aminobenzoate), adjusted to pH7.5 with NaHCO<sub>3</sub> and gassed with air. After cessation of breathing movements, fish were placed onto an operating table that allowed continuous irrigation of the gills. An indwelling cannula (Clay Adams PE 50 polyethylene tubing: internal diameter 0.580mm, outer diameter 0.965mm) was implanted into the dorsal aorta (Soivio *et al.* 1975). After surgery, the fish were allowed to recover for 48h in individual opaque boxes supplied with aerated tapwater.

After recovery, blood samples (0.5ml) were taken before (pre) and 2, 5 and 10min after injection of different doses (50, 100 or 250nmolkg<sup>-1</sup>) of serotonin, into different groups of fish; control fish were injected with saline. In addition, one group of fish was pre-treated with the serotonergic receptor antagonist methysergide (1.5mgkg<sup>-1</sup>) before serotonin (100nmolkg<sup>-1</sup>) was injected. In all fish a final sample was taken 1h after the 10min blood sample (recovery). The blood was centrifuged (1min at 12000g) and the plasma was immediately removed, frozen in liquid nitrogen and stored at -80°C until catecholamine levels could be determined. Analysis was carried out within one week of sampling in all experiments. The red blood cells were suspended in Cortland saline (Wolf, 1963) and re-injected into the dorsal aortic cannula after each sampling.

#### *In situ experiments*

The fish were killed by a sharp blow to the head and immediately injected with 1500i.u. of heparin (0.1ml) *via* the caudal vein. An incision was made ventrally and the left side of the body wall was cut away to expose the swimbladder and underlying kidney. The right posterior cardinal vein was cannulated as far back as possible, approximately two-thirds along the length of the kidney, using polyethylene tubing (PE 160) for the inflow of perfusion fluid and with a ligature secured around the entire fish to prevent leakage from the inflow cannula. The heart was exposed, the bulbus arteriosus was cannulated, and the cannula was fed into the ventricle for collection of the outflowing perfusate. The fish was placed on ice and the tissues were kept moist with Cortland saline. The posterior cardinal vein was perfused at a constant flow (1.0mlmin<sup>-1</sup>) with modified Cortland saline (125mmol l<sup>-1</sup> NaCl, 2mmol l<sup>-1</sup> KCl, 2mmol l<sup>-1</sup> MgSO<sub>4</sub>, 5mmol l<sup>-1</sup> NaHCO<sub>3</sub>, 7.5mmol l<sup>-1</sup> glucose, 2.0mmol l<sup>-1</sup> CaCl<sub>2</sub>, 1.25mmol l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>) using a cardiac pump at a frequency of 40strokesmin<sup>-1</sup>. The saline was gassed with 0.5% CO<sub>2</sub> in air using a Wösthoff gas mixing pump. The pressure pulse was damped by installing a windkessel on the perfusion pump outflow. Effluent perfusion fluid was collected in microcentrifuge tubes at 1min intervals using a fraction collector. All samples were immediately frozen in liquid nitrogen and then stored at -80°C subsequent to analysis of catecholamine or serotonin levels.

*Series 1: characterization of the preparation*

The head kidney preparation was perfused for at least 20min before commencing the experiment. The experiment was initiated by collecting the outflowing perfusate, ( $1\text{mlmin}^{-1}$ , in 1ml fractions), for 2min. At the end of these two 'pre' samples, different doses ( $10^{-4}$ ,  $10^{-5}$ ,  $5 \times 10^{-6}$ ,  $10^{-6}$ ,  $10^{-7}$  mol  $\text{kg}^{-1}$ ) of the cholinergic agonist carbachol, or saline (control), were injected as a single bolus (0.3ml) and the perfusate was collected each minute for another 6min. This allowed for the establishment of a dose-response relationship for catecholamine release. Each preparation was subjected to a single dose of carbachol.

In another set of experiments, the ganglionic nicotinic receptor antagonist hexamethonium was added to the perfusion fluid (final concentration  $10^{-4}$  mol  $\text{l}^{-1}$ ) and the preparation was pre-perfused for 30min before the experiment started. After collecting the perfusate for 2min, a bolus injection of  $10^{-5}$  mol  $\text{kg}^{-1}$  carbachol was injected and the perfusate was collected for another 6min.

The  $\text{Ca}^{2+}$  dependence of catecholamine release was tested by perfusing a set of preparations with  $\text{Ca}^{2+}$ -free saline. After sample 2, a bolus of  $10^{-5}$  mol  $\text{kg}^{-1}$  carbachol was injected and the perfusate was collected for another 6min.

*Series 2: effects of serotonin on catecholamine release*

Three separate experimental series were performed in which the perfusion fluid was varied. In the first, the saline used previously (see above) was not modified. In the second, hexamethonium (final concentration  $10^{-4}$  mol  $\text{l}^{-1}$ ) was added and, in the third, the serotonergic receptor antagonist methysergide (final concentration  $10^{-5}$  mol  $\text{l}^{-1}$ ) was added. In all cases the preparations were pre-perfused for 30min before starting the experiment. Serotonin ( $250\text{nmolkg}^{-1}$ ) was injected into the inflowing saline after sample 2 and the preparation was then perfused for another 6min.

*Series 3: storage of serotonin and the effects of cholinergic stimulation on serotonin release*

Six fish were killed with a sharp blow to the head and the right posterior cardinal vein was removed, weighed and placed in 1ml of perchloric acid (4%) containing  $2\text{mgml}^{-1}$  EDTA/ $0.5\text{mgml}^{-1}$  sodium bisulphate. The tissue was then sonicated for 20s to ensure complete cellular disruption. The sample was centrifuged (10min,  $12000g$ ) and the supernatant was removed and kept frozen at  $-80^{\circ}\text{C}$  prior to analysis of serotonin levels.

Carbachol ( $10^{-5}$  mol  $\text{kg}^{-1}$ ) was injected into the inflowing saline in six perfused head kidney preparations after sample 2, and the outflow was collected for another 6min. These samples were later analyzed for serotonin levels.

*Analytical procedures*

Plasma noradrenaline and adrenaline levels were determined on alumina-extracted plasma or saline samples using high performance liquid chromatography (HPLC) with electrochemical detection, according to the basic method of Woodward (1982). Serotonin levels in tissue or saline were also determined by using HPLC (same system as for the

catecholamines), although the samples were not subjected to alumina extraction; the flow of the mobile phase was increased from 1.0 to 1.5 ml min<sup>-1</sup>.

#### *Statistical analysis*

Statistical analyses were performed using the Wilcoxon signed-rank sum test, and differences where  $P \leq 0.05$  were considered significant. When variables were used in more than one paired comparison (a maximum of three comparisons were made) in the statistical evaluation, a sequentially rejective Bonferroni test (Holm, 1979) was used to eliminate, as far as possible, the risk of discarding any true null hypothesis.

The following comparisons were made: the values immediately before injecting carbachol (PRE) were compared with the values 3 min after injection. Also, the values 3 min after injection of carbachol when hexamethonium was present or Ca<sup>2+</sup> was removed were compared with the same values from preparations perfused with normal saline.

The values immediately before injecting serotonin (PRE) were compared with the values for adrenaline 5 min after serotonin injection and the values for noradrenaline 1 min after serotonin injection. The times were chosen to reflect the temporal differences in release of the two catecholamines induced by serotonin. The 5 and 1 min values, respectively, were compared between the preparations perfused with normal saline and the ones containing hexamethonium or methysergide.

For the *in vivo* experiments, the PRE injection value was compared with the peak value (2 min after injection) for both catecholamines.

## **Results**

### *In vivo experiments*

The plasma levels of noradrenaline and adrenaline after intra-arterial injection of serotonin (50, 100 or 250 nmol kg<sup>-1</sup>) rose to similar levels to those found in a previous preliminary study (Fritsche *et al.* 1992). Following injection of 100 nmol kg<sup>-1</sup> serotonin, adrenaline peaked at a concentration of approximately 10 nmol l<sup>-1</sup> and noradrenaline at approximately 8 nmol l<sup>-1</sup> after 2 min (Fig. 1). Both catecholamines had returned to resting (PRE) values 1 h after injection of 100 nmol kg<sup>-1</sup>. Pre-treatment with the general serotonergic antagonist methysergide did not abolish the serotonin-induced increase in plasma catecholamine levels (Fig. 2). The repeated blood sampling had no effect on plasma catecholamine levels, as confirmed by injecting saline into one group (Fig. 1).

### *In situ experiments*

#### *Series 1: characterization and validation of the preparation*

Injections of different doses of the cholinergic receptor agonist carbachol (10<sup>-7</sup>, 10<sup>-6</sup>, 5 × 10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-4</sup> mol kg<sup>-1</sup>) resulted in a significant (10<sup>-6</sup>, 5 × 10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-4</sup> mol kg<sup>-1</sup>) and dose-dependent release of both adrenaline and noradrenaline (Fig. 3). The response was largest at 10<sup>-5</sup> mol kg<sup>-1</sup> and tended to decline at 10<sup>-4</sup> mol kg<sup>-1</sup>, especially for noradrenaline. Adrenaline was the predominant

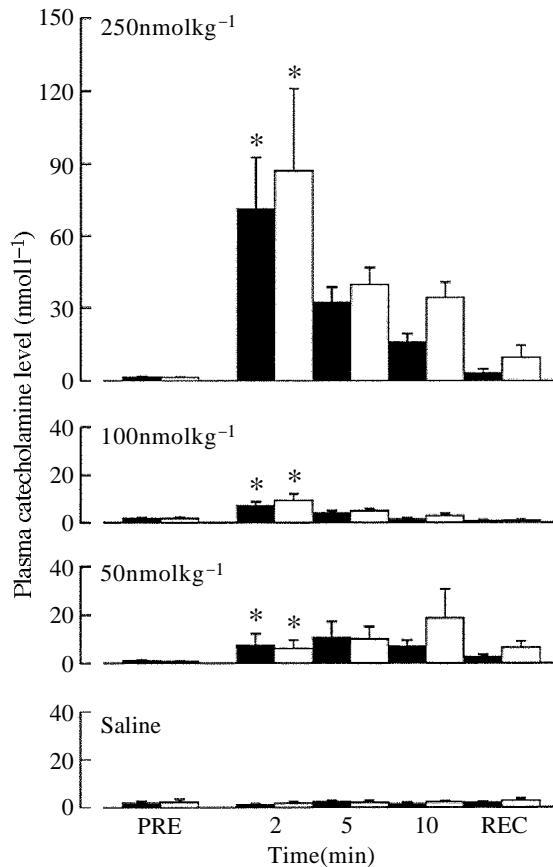


Fig. 1. Plasma catecholamine levels (means + 1 s.e.m.;  $N=7$ ), before (PRE) and 2, 5, 10 and 60min (recovery, REC) after injection of different doses (50, 100, 250nmolkg<sup>-1</sup>) of serotonin or saline (control). \* indicates a statistically significant difference compared with the corresponding pre-injection value. Open columns, adrenaline level; filled columns, noradrenaline level.

catecholamine released at all doses. Since a dose of carbachol of 10<sup>-5</sup>molkg<sup>-1</sup> elicited clear and significant increases in both adrenaline and noradrenaline, this dose was chosen for all subsequent experiments.

The presence of hexamethonium in the perfusion fluid abolished the carbachol (10<sup>-5</sup>molkg<sup>-1</sup>)-induced increase in noradrenaline but not in adrenaline (Fig. 4). Injection of carbachol (10<sup>-5</sup>molkg<sup>-1</sup>) to preparations perfused with Ca<sup>2+</sup>-free saline caused no increase in adrenaline or noradrenaline levels.

#### *Series 2: direct effects of serotonin*

A bolus injection of serotonin (250nmolkg<sup>-1</sup>) to the perfusion fluid caused significant increases in both noradrenaline and adrenaline levels (Fig. 5). There were temporal differences in the release of the two catecholamines. Noradrenaline showed maximum release 1min and adrenaline 5min after injection.

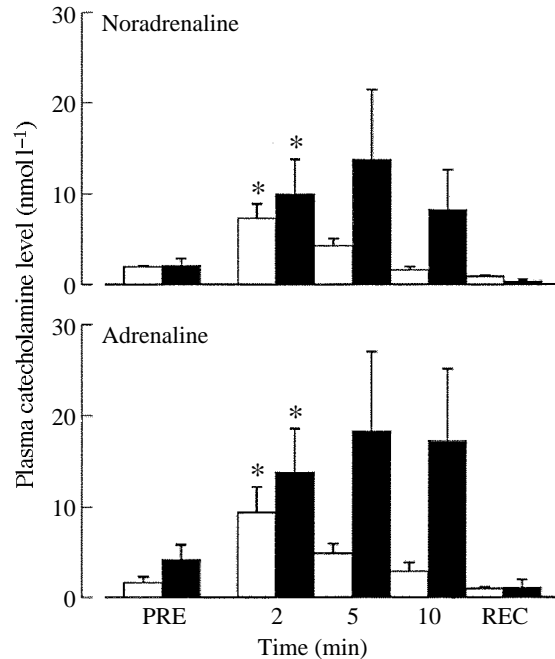


Fig. 2. Plasma catecholamine levels (means + 1 S.E.M.;  $N=7$ ) before (PRE) and 2, 5, 10 and 60min (recovery, REC) after injection of  $100\text{nmolkg}^{-1}$  serotonin into untreated animals (open bars) and in animals pre-treated with methysergide (filled bars). \* indicates a statistically significant difference compared with the corresponding pre-injection value.

Addition of hexamethonium to the perfusion fluid had no effect on the serotonin-induced release of either adrenaline or noradrenaline. However, the presence of methysergide in the perfusion fluid blocked the serotonin-induced release of adrenaline, but not of noradrenaline (Fig. 5).

### Series 3: storage of serotonin and effects of cholinergic stimulation on serotonin release

The tissue extract from the posterior cardinal vein contained large quantities of serotonin:  $44.6 \pm 5.96 \mu\text{g g}^{-1}$  tissue ( $N=7$ ). Bolus injections of carbachol ( $10^{-5} \text{mol kg}^{-1}$ ) to the inflowing perfusate did not evoke any release of serotonin ( $N=6$ ; data not shown).

## Discussion

### In vivo experiments

In agreement with a previous preliminary study (Fritsche *et al.* 1992), we demonstrate dose-dependent increases in the levels of plasma catecholamines after intra-arterial administration of serotonin *in vivo*. Few comparable studies have been performed in other vertebrate groups, although the injection of serotonergic receptor agonists into rats (Chaouloff *et al.* 1992) elicits catecholamine release from the adrenal gland, the functional counterpart to the head kidney of teleost fish. It has been suggested that the serotonergic receptors are located centrally and the central sites of action of specific

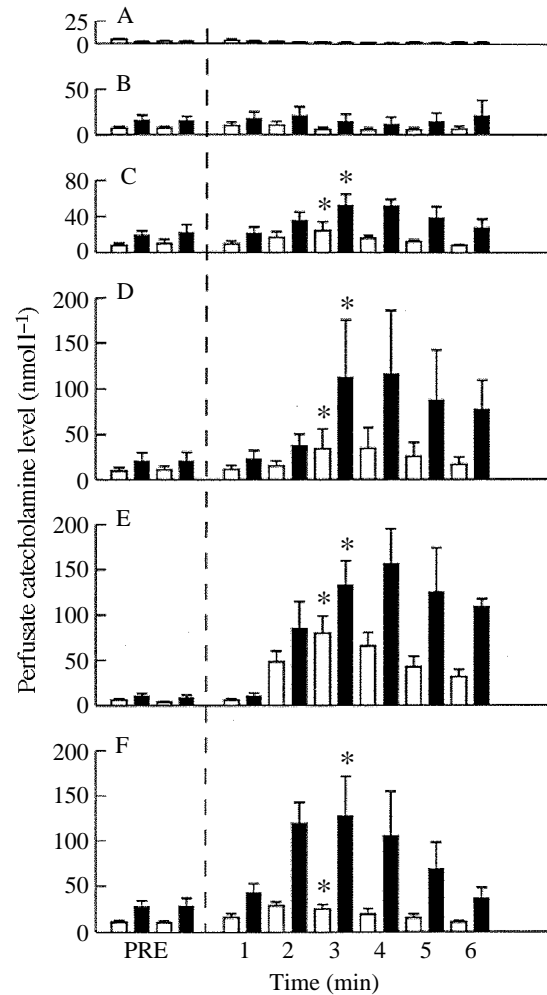


Fig. 3. Catecholamine release (filled bars, adrenaline; open bars, noradrenaline) from an *in situ* perfused head kidney preparation, before (PRE) and after a bolus injection of (A) saline or different doses (B)  $10^{-7}$ , (C)  $10^{-6}$ , (D)  $5 \times 10^{-6}$ , (E)  $10^{-5}$ , (F)  $10^{-4}$  mol kg<sup>-1</sup> carbachol. Values are shown as means + 1 S.E.M.,  $N=7$ . \* indicates a statistically significant difference from the pre-injection value.

5-HT<sub>1a</sub>, 5-HT<sub>1c</sub> and 5-HT<sub>2</sub> receptor agonists have been clearly demonstrated (Bagdy *et al.* 1989a,b; Laude *et al.* 1990). The serotonin-induced release of catecholamines in the trout could thus be centrally mediated and involve the triggering of reflex-release from the chromaffin cells. Additional mechanisms could involve a serotonin-induced hypoxaemia (Fritsche *et al.* 1992) or direct interaction of serotonin with the chromaffin cells. The specific stimulatory effect of blood hypoxaemia on chromaffin tissue catecholamine release was demonstrated recently in a blood-perfused head kidney preparation of Atlantic cod (*Gadus morhua*; Perry *et al.* 1991). The profound blood hypoxaemia accompanying the injection of serotonin is completely abolished after pre-



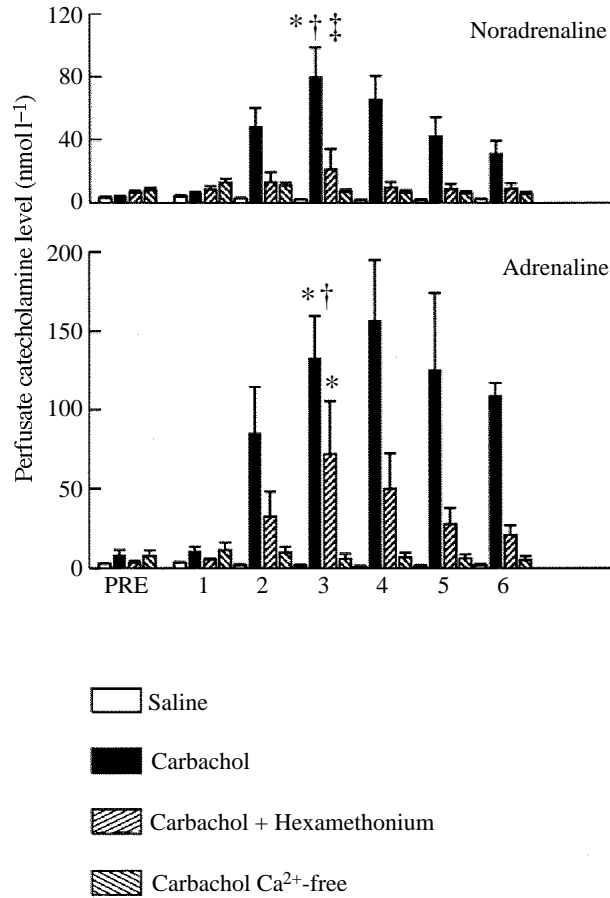


Fig. 4. Perfusate catecholamine levels before (PRE) and 1, 2, 3, 4, 5 and 6 min after injection of saline (open bars) or carbachol ( $10^{-5}$  mol kg $^{-1}$ ) into preparations perfused with normal saline (filled bars), saline containing hexamethonium (stippled bars rising right) or with saline where the  $Ca^{2+}$  had been removed (stippled bars rising left). Values are shown as means  $\pm$ 1 S.E.M.,  $N=7$ . \* indicates a statistically significant difference compared with the corresponding pre-injection value. † indicates a statistically significant difference compared with the corresponding time in the  $Ca^{2+}$ -free group and ‡ from the corresponding time in the hexamethonium group.

treatment of the fish with the general serotonergic receptor antagonist methysergide (Fritsche *et al.* 1992). These findings enabled us to use methysergide as a tool in the present study to prevent the hypoxaemia. It is clear from the experimental results that the serotonin-induced hypoxaemia is not solely responsible for the increase in plasma catecholamine levels after injections of serotonin.

#### In situ experiments

##### Series 1: characterization and validation of the preparation

The saline-perfused chromaffin cells responded to the cholinergic receptor agonist

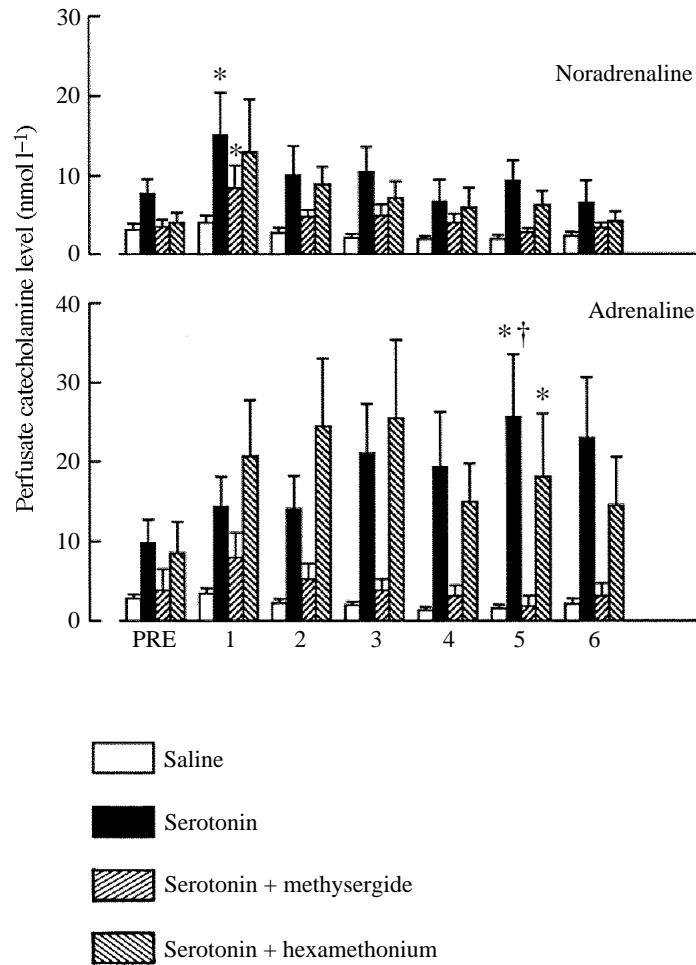


Fig. 5. Perfusate catecholamine levels before (PRE) and 1, 2, 3, 4, 5 and 6 min after injection of saline (open bars) or serotonin ( $250\text{nmolkg}^{-1}$ ) in preparations perfused with normal saline (filled bars), saline containing methysergide (stippled bars rising right) or saline containing hexamethonium (stippled bars rising left). Values are shown as means  $\pm 1$  S.E.M.,  $N=7$ . \* indicates a statistically significant difference compared with the corresponding pre-injection value. † indicates a statistically significant difference compared to the corresponding time in the group perfused with saline containing methysergide.

carbachol by releasing noradrenaline and adrenaline in a dose-dependent fashion. However, adrenaline was the predominant catecholamine released at all injected doses of carbachol. This probably reflects the storage ratio of these two catecholamines (75% adrenaline; 25% noradrenaline; S. G. Reid and S. F. Perry, unpublished observations). A similar situation prevails in the Atlantic cod (*Gadus morhua*), where the storage ratio is 86% adrenaline and 14% noradrenaline (Abrahamsson and Nilsson, 1976). If storage level were the sole determinant of release level, one would predict that adrenaline levels would always exceed noradrenaline levels upon stimulation of the chromaffin tissue; this is clearly not the case (for a review, see Randall and Perry, 1993). For example, in the

Atlantic cod, noradrenaline is the predominant catecholamine released during rapidly induced hypoxia (Fritsche and Nilsson, 1989), whereas adrenaline release dominates when hypoxia is induced more slowly (Kinkead *et al.* 1991). The reason for these methodology-dependent differences in catecholamine release are unclear, but they could be related to different stimuli triggering the release according to the nature of the imposed stress (i.e. a neuronal-dependent *versus* a humoral-dependent mechanism). The fact that the ratio between the two catecholamines released varies with different stimuli suggests that they are stored in different chromaffin cell types, as has been demonstrated in amphibians (Coupland 1971; Mastrolia *et al.* 1976; Delarue *et al.* 1988, 1992) and mammals (Holzwarth and Brownfield, 1985; Holzwarth *et al.* 1984; Brownfield *et al.* 1985). In support of this idea, we have recently demonstrated profoundly different ratios of released catecholamines in a saline-perfused head kidney preparation of rainbow trout, depending on the nature of the releasing stimulus used (e.g. cholinergic receptor stimulation *versus* non-specific depolarization using 60mmol l<sup>-1</sup> KCl) (S. G. Reid and S. F. Perry, unpublished observations).

The finding that the nicotinic receptor antagonist hexamethonium blocks the release of noradrenaline completely, but not that of adrenaline, provides further evidence for at least two different cell types storing catecholamines in the trout. The data suggest that nicotinic stimulation causes release of noradrenaline alone or in combination with adrenaline (since the presence of hexamethonium in the perfusion fluid reduces the amount of adrenaline released). Since carbachol (which stimulates both nicotinic and muscarinic receptors) elicits adrenaline release in the presence of hexamethonium, the existence of muscarinic receptors on the adrenaline-storing cells is possible. This resembles the situation in the cat adrenal gland, where nicotinic agonists have been shown to stimulate the release of both adrenaline and noradrenaline, whereas muscarinic agonists preferentially elicit the release of adrenaline (Michelena *et al.* 1991). The physiological data indicating differential release of adrenaline and noradrenaline in mammals are consistent with histological studies showing that the two catecholamines are localized in different cell types (Chritton *et al.* 1991).

Irrespective of the stimulus for release, the exocytosis of stored catecholamines is dependent upon the depolarization of the cells, the activation of voltage-dependent Ca<sup>2+</sup> channels and the subsequent influx of Ca<sup>2+</sup> (Chritton *et al.* 1991; Burgoyne, 1991; Augustine and Neher, 1992). The exocytotic release of catecholamines from the chromaffin cells of trout is also Ca<sup>2+</sup>-dependent. The data suggest the existence of similar control and storage mechanisms in the trout to those in mammalian chromaffin cells.

#### *Series 2: effects of serotonin on catecholamine release*

The results of the present study clearly demonstrate a direct stimulatory effect of serotonin on the chromaffin cells. The release of both adrenaline and noradrenaline was evoked by application of serotonin to the saline-perfused head kidney preparation, although there were temporal differences with respect to the timing of the maximal response. These temporal differences also support the existence of at least two different populations of chromaffin cells. A direct effect of serotonin in stimulating catecholamine release from the chromaffin cells of fish has not, to our knowledge, been described before.

Hexamethonium did not abolish the serotonin-induced release of either adrenaline or noradrenaline, suggesting that serotonin does not act through cholinergic nicotinic receptors. The presence of methysergide in the perfusion fluid completely blocked the serotonin-induced release of adrenaline without significantly affecting the release of noradrenaline. We suggest, therefore, that serotonergic methysergide-sensitive receptors exist on the adrenaline-containing chromaffin cells, and these, when stimulated, trigger the preferential release of adrenaline. The reason for the differences between the *in vivo* and the *in situ* response, where methysergide only blocks the serotonin-induced release of catecholamines *in situ*, is not fully understood. However, serotonin is known to have a variety of effects *in vivo*, such as the triggering of nervous reflexes (mediated by methysergide-insensitive receptors) which, in turn, could cause catecholamine release (Marwood and Stokes, 1984; Vanhoutte, 1986; Fritsche and Nilsson, 1993).

*Series 3: storage of serotonin and the effects of cholinergic stimulation on serotonin release*

This is the first study to report the presence of serotonin in the posterior cardinal vein (PCV) of a teleost fish. Traditionally, the chromaffin cells have been considered as a store primarily for catecholamines. However, immunocytochemical studies have revealed serotonin immunoreactivity in mammalian (Brownfield *et al.* 1985; Lefebvre *et al.* 1992) as well as in amphibian (Delarue *et al.* 1988) chromaffin cells. Tissue extracts from the PCV contain large quantities of serotonin ( $44.6 \pm 6.0 \mu\text{g g}^{-1}$  tissue), that are comparable with the levels of stored catecholamines ( $38.8 \pm 14.4 \mu\text{g g}^{-1}$  adrenaline and  $9.9 \pm 3.9 \mu\text{g g}^{-1}$  noradrenaline S. G. Reid and S. F. Perry, unpublished observations). As a comparison, the stored level of serotonin in frog chromaffin tissue is only  $580 \text{ng g}^{-1}$  tissue; this value represents 0.02% of the stored adrenaline (Delarue *et al.* 1988).

The fact that splanchnectomized frogs showed increased immunoreactivity to serotonin suggests that the release of serotonin from chromaffin cells may be triggered by cholinergic stimulation (Delarue *et al.* 1988). The teleost chromaffin tissue is innervated by an extrinsic nerve supply *via* myelinated, presumably preganglionic, fibres that pass through the sympathetic chain ganglion corresponding to the third spinal nerve and the 'satellite ganglion' to enter the PCV (Nilsson, 1983). The finding that carbachol did not evoke any serotonin release from the trout perfused head kidney preparation indicates that the release is not triggered by cholinergic stimulation and/or that the serotonin is not stored in the chromaffin cells. The high levels of serotonin measured in the tissue extracts might arise from nerve fibres in the posterior cardinal vein, although this would be different from the situation in the amphibian adrenal gland, which lacks serotonergic nerve fibres (Delarue *et al.* 1988). Another possibility is a distinct serotonin-storing cell type devoid of nicotinic or muscarinic receptors. Regardless of where serotonin is stored, it could be released into the PCV in the area of chromaffin cells during stress to augment the usual cholinergic release mechanism. Whether this strategy is used by fish to 'fine-tune' the release of the two catecholamines remains to be determined.

This study was supported by grants from the Swedish Natural Science Research Council and the Helge Axelsson-Johnsson foundation to R.F. and by NSERC Operating,

Equipment and Scientific Exchange grants to S.F.P. and by CNRS grants to S.T. We wish to thank Mr Koushyar Keyhan for excellent technical assistance with the catecholamine analysis.

### References

- ABRAHAMSSON, T. AND NILSSON, S. (1976). Phenylethanolamine-*N*-methyl transferase (PNMT) activity and catecholamine content in chromaffin tissue and sympathetic neurons in the cod, *Gadus morhua*. *Acta physiol. scand.* **96**, 94–99.
- AUGUSTINE, G. J. AND NEHER, E. (1992). Calcium requirements for secretion in bovine chromaffin cells. *J. Physiol., Lond.* **450**, 247–271.
- BAGDY, G., CALOGERO, A. E., MURPHY, D. L. AND SZEMEREDI, K. (1989a). Serotonin agonists cause parallel activation of the sympathoadrenomedullary system and the hypothalamo-pituitary-adrenocortical axis in conscious rats. *Endocrinology* **125**, 2664–2669.
- BAGDY, G., SZEMEREDI, K., KANYICSKA, B. AND MURPHY, D. L. (1989b). Different serotonin receptors mediate blood pressure, heart rate, plasma catecholamine and prolactin responses to *m*-chlorophenylpiperazine in conscious rats. *J. Pharmac. exp. Ther.* **250**, 72–78.
- BROWNFIELD, M. S., POFF, B. C. AND HOLZWARTH, M. A. (1985). Ultrastructural immunocytochemical co-localization of serotonin and PNMT in adrenal medullary vesicles. *Histochemistry* **83**, 41–46.
- BURGOYNE, R. D. (1991). Control of exocytosis in adrenal chromaffin cells. *Biochim. biophys. Acta* **1071**, 174–202.
- CHAOULOFF, F., GUNN, S. H. AND YOUNG, J. B. (1991). Influence of 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptor antagonists on insulin-induced adrenomedullary catecholamine release. *Neuroendocrinology* **54**, 639–645.
- CHAOULOFF, F., GUNN, S. H. AND YOUNG, J. B. (1992). Central 5-hydroxytryptamine<sub>2</sub> receptors are involved in the adrenal catecholamine-releasing and hyperglycemic effects of the 5-hydroxytryptamine indirect agonist *d*-Fenfluramine in the conscious rat. *J. Pharmac. exp. Ther.* **260**, 1008–1016.
- CHRITTON, S. L., DOUSA, M. K., YAKSH, T. L. AND TYCE, G. M. (1991). Nicotinic and muscarinic evoked release of canine adrenal catecholamines and peptides. *Am. J. Physiol.* **260**, R589–R599.
- COUPLAND, R. E. (1971). Observations on the form and size distribution of chromaffin granules and on the identity of adrenaline- and noradrenaline-storing chromaffin cells in vertebrates and man. *Mem. Soc. Endocr.* **19**, 611–635.
- DELARUE, C., BECQUET, D., IDRES, S., HERY, F. AND VAUDRY, H. (1992). Serotonin synthesis in adrenochromaffin cells. *Neuroscience* **46**, 495–500.
- DELARUE, C., LEBOULENGER, F., MORRA, M., HÉRY, F., VERHOFSTAD, A. J., BÉROD, A., DENOROY, L., PELLETIER, G. AND VAUDRY, H. (1988). Immunohistochemical and biochemical evidence for the presence of serotonin in amphibian adrenal chromaffin cells. *Brain Res.* **459**, 17–26.
- FRANZONI, M., BELTRAMO, M. L., SAPEI, C. AND CALAIS, D. (1987). Direct simultaneous visualization of GABA innervation and serotonin uptake in adrenal medullary cells. *Soc. Neurosci. Abstr.* **13**, 213.3.
- FRITSCHÉ, R. AND NILSSON, S. (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Expl Biol.* **48**, 153–160.
- FRITSCHÉ, R. AND NILSSON, S. (1993). Cardiovascular and respiratory control during hypoxia. In *Ecophysiology*, chapter 7 (ed. C. Rankin and F. B. Jensen), pp. 180–199. London, New York: Chapman and Hall.
- FRITSCHÉ, R., THOMAS, S. AND PERRY, S. F. (1992). Effects of serotonin on circulation and respiration in the rainbow trout, *Oncorhynchus mykiss*. *J. exp. Biol.* **173**, 59–73.
- HATHAWAY, C. B., BRINN, J. E. AND EPPLE, A. (1989). Catecholamine release by catecholamines in the eel does not require the presence of brain or anterior spinal cord. *J. exp. Zool.* **249**, 338–342.
- HOLM, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70.
- HOLZWARTH, M. A. AND BROWNFIELD, M. S. (1985). Serotonin coexists with epinephrine in rat adrenal medullary cells. *Neuroendocrinology* **41**, 230–236.
- HOLZWARTH, M. A., SAWETAWAN, C. AND BROWNFIELD, M. S. (1984). Serotonin immunoreactivity in the adrenal medulla: distribution and response to pharmacological manipulation. *Brain Res. Bull.* **13**, 299–308.

- JÖNSSON, A.-C., WAHLQUIST, I. AND HANSSON, T. (1983). Effects of hypophysectomy and cortisol on the catecholamine biosynthesis and catecholamine content in chromaffin tissue from rainbow trout, *Salmo gairdneri*. *Gen. comp. Endocr.* **51**, 278–285.
- KINKEAD, R., FRITSCHÉ, R., PERRY, S. F. AND NILSSON, S. (1991). The role of circulating catecholamines in the ventilatory and hypertensive responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Physiol. Zool.* **64**, 1087–1109.
- LAUDE, D., BAUDRIE, V., MARTIN, G. R. AND CHAULOUFF, F. (1990). Effects of the 5-HT<sub>1</sub> receptor agonist DP-5-CT, CGS 12066B and RU 24969 on plasma adrenaline and glucose levels in the rat. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.* **342**, 378–381.
- LEFEBVRE, H., CONTESSE, V., DELARUE, C., FEUILLOLEY, M., HERY, F., GRISE, P., RAYNAUDS, G., VERHOFSTAD, A. A. J., WOLF, L. M. AND VAUDRY, H. (1992). Serotonin-induced stimulation of cortisol secretion from human adrenocortical tissue is mediated through activation of a serotonin<sub>4</sub> receptor subtype. *Neuroscience* **47**, 999–1007.
- MARWOOD, J. F. AND STOKES, G. S. (1984). Serotonin (5-HT) and its antagonists: involvement in the cardiovascular system. *Clin. exp. Pharmacol. Physiol.* **11**, 439–456.
- MASTROLIA, L., GALLO, V. AND MANELLI, H. (1976). Cytological and histochemical observations on chromaffin cells of the suprarenal gland of *Triturus cristatus* (urodele amphibian). *Boll. Zool.* **43**, 27–36.
- MICHELENA, P., MORO, M. A., CASTILLO, C. J. F. AND GARCIA, A. G. (1991). Muscarinic receptors in separate populations of noradrenaline and adrenaline containing chromaffin cells. *Biochem. Biophys. Res. Commun.* **177**, 913–919.
- NILSSON, S. (1983). *Autonomic Nerve Function in the Vertebrates*. Berlin, New York: Springer Verlag.
- OPDYKE, D. F., BULLOCK, J., KELLER, N. E. AND HOLMES, K. (1983a). Dual mechanism for catecholamine secretion in the dogfish shark *Squalus acanthias*. *Am. J. Physiol.* **244**, R641–R645.
- OPDYKE, D. F., BULLOCK, J., KELLER, N. E. AND HOLMES, K. (1983b). Effect of ganglionic blockade on catecholamine secretion in exercised dogfish. *Am. J. Physiol.* **245**, R915–R919.
- PERRY, S. F., FRITSCHÉ, R., KINKEAD, R. AND NILSSON, S. (1991). Control of catecholamine release *in vivo* and *in situ* in the Atlantic cod, *Gadus morhua* during hypoxia. *J. exp. Biol.* **155**, 549–566.
- RANDALL, D. J. AND PERRY, S. F. (1993). Catecholamines. In *Fish Physiology* vol. 12B, *The Cardiovascular System* (ed. D. J. Randall and W. S. Hoar), pp. 255–300. New York: Academic Press.
- SOIVIO, A., NYHOLM, K. AND WESTMAN, K. (1975). A technique for repeated blood sampling of the blood of individual fish. *J. exp. Biol.* **62**, 207–217.
- VANHOUTTE, P. M. (1986). Serotonin, adrenergic nerves, endothelial cells and vascular smooth muscle. *Prog. appl. Microcirc.* **10**, 1–11.
- VERHOFSTAD, A. A. J. AND JÖNSSON, G. (1983). Immunohistochemical and neurochemical evidence for the presence of serotonin in the adrenal medulla of the rat. *Neuroscience* **10**, 1443–1453.
- WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progve Fish Cult.* **25**, 135–140.
- WOODWARD, J. J. (1982). Plasma catecholamines in resting rainbow trout, *Salmo gairdneri* Richardson, by high pressure liquid chromatography. *J. Fish Biol.* **21**, 429–432.