The Journal of Experimental Biology 213, 1813-1821

doi:10.1242/jeb.035444

Homeostasis of glucose in the rainbow trout (*Oncorhynchus mykiss* Walbaum): the role of serotonin

Rosa I. Caamaño Tubío, Jorge Pérez-Maceira and Manuel Aldegunde*

Laboratorio de Fisiología Animal (Instituto de Acuicultura), Facultad de Biología, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

*Author for correspondence (manuelalejo.aldegunde@usc.es)

Accepted 15 February 2010

SUMMARY

In this study, we evaluated, for the first time, the 5-HT (serotonin)-mediated control of glucose homeostasis in the rainbow trout *Oncorhynchus mykiss*. Intraperitoneal administration of 5-HT increased plasma levels of glucose, adrenaline and noradrenaline. By contrast, intracerebroventricular administration of 5-HT did not cause any significant variation in plasma levels of glucose. The release of endogenous 5-HT following intraperitoneal administration of d-fenfluramine led to a significant increase in plasma levels of glucose and adrenaline. Intraperitoneal administration of (1) MIAN (a 5-HT2 receptor antagonist) did not block either the hyperglycaemic action or the increase in plasma levels of adrenaline induced by 5-HT, but did block the increase in plasma levels of noradrenaline, and (2) 5-CT (a 5-HT1 agonist) increased the plasma levels of glucose and of adrenaline, without altering those of noradrenaline. Administration of TFMPP (a 5-HT1B agonist) did not increase the plasma levels of glucose, and the hyperglycaemic action of 5-HT was not blocked by antagonists of 5-HT1A (WAY 100635), 5-HT1D (BRL 15572), 5-HT2B (SB 204741) or 5-HT7 (pimozide) receptors. It was demonstrated that, in rainbow trout, peripheral 5-HT, but not brain 5-HT intervenes in the modulation of glucose homeostasis with a hyperglycaemic effect. This effect is associated with the release of adrenaline and activation of 5-HT1-like receptors. As far as could be determined in the present study, these 5-HT1-like receptors are unrelated to either the 5-HT1A, 5-HT1B or 5-HT1D receptor subtypes of mammals. The 5-HT2-type receptors may mediate the release of noradrenaline, but not of adrenaline, and furthermore, do not appear to play an important role in the hyperglycaemic effect exerted by 5-HT.

Key words: glucose, serotonin, serotonin receptors, catecholamines.

INTRODUCTION

Studies performed in rodents suggest that 5-hydroxytryptamine (5-HT; serotonin) plays a role in the regulation of blood glucose. Thus, it has been reported that in rodents the injection of 5-HT or 5-HT receptor agonists results in hyperglycaemia (Chaouloff and Jeanrenaud, 1987; Yamada et al., 1995; Sugimoto et al., 1996a; Sugimoto et al., 1996b; Yamada et al., 1997). The 5-HT receptors that appear to mediate this hyperglycaemic action are the central 5-HT1A subtype (Uvnäs-Moberg et al., 1996; Sugimoto et al., 2001) and/or the central or peripheral 5-HT2 (Chaouloff et al., 1990b; Yamada et al., 1995; Yamada et al., 1997; Sugimoto et al., 1996a; Sugimoto et al., 1996b). However, the exact nature of the role that 5-HT plays in controlling blood glucose levels has still to be determined. Although some authors report that the effect of 5-HT appears to be related to changes in the plasma level of insulin (Chaouloff and Jeanrenaud, 1987; Uvnäs-Moberg et al., 1996; Yamada et al., 1998), others suggest that the effect is insulin independent (Chaouloff and Jeanrenaud, 1987; Yamada et al., 1997). Furthermore, according to some authors, it is the brain 5-HT that is involved in hyperglycaemia (Sugimoto et al., 1996a; Chaouloff et al., 1990a; Chaouloff et al., 1992), whereas others state that it is the peripheral 5-HT that is involved (Yamada et al., 1995; Sugimoto et al., 1996b; Yamada et al., 1997; Chaouloff et al., 1990b). Notwithstanding, the hyperglycaemia associated with activation of 5-HT receptors, whether central (5-HT1A and/or 5-HT2) or peripheral (5-HT2), is always associated with release of adrenaline (Yamada et al., 1995; Sugimoto et al., 1996b; Chaouloff et al., 1990b).

Although the rainbow trout ingests relatively small amounts of carbohydrates compared with other species and possesses a limited capacity to utilize dietary carbohydrate for energy purposes, the use of glucose as an oxidative substrate is essential for the functioning of the brain, and therefore the fish need a continuous supply of glucose for the blood (Aldegunde et al., 2000; Soengas and Aldegunde, 2002). Despite this, the mechanisms that control glucose homeostasis in fish have not been clarified, although there is some evidence that catecholamines (adrenaline and noradrenaline) induce a marked hyperglycaemic effect (Reid et al., 1998; Iwama et al., 2004), in a similar way to that observed in other vertebrates. In the rainbow trout, secretion of catecholamines is largely mediated by the release of acetylcholine from cholinergic preganglionic fibres of the sympathetic nerves, and subsequent activation of the nicotinic and muscarinic receptors present in the chromaffin cells (Montpetit and Perry, 1999). However, and as in other vertebrates, other mechanisms mediated by non-cholinergic neurotransmitters also modulate the secretion of catecholamines. It has been observed that 5-HT may induce the release of catecholamines from the chromaffin cells in both rainbow trout (Reid et al., 1998; Fritsche et al., 1993) and in the Atlantic hagfish (Bernier and Perry, 1996).

At present, nothing is known about the possible role of 5-HT in glucose homeostasis in fish. However, we can take the following into consideration: (1) the existence of serotoninergic modulation of catecholamine release; (2) in fish, 5-HT is involved in the physiological and behavioural responses to situations of stress associated with hyperglycaemia (Blanchard et al., 1993; Winberg and Nilsson, 1993); and (3) the abundance of peripheral 5-HT in

the rainbow trout (Caamaño-Tubio et al., 2007). All of this appears to indicate that 5-HT may play an important role in the modulation of glucose homeostasis in fish. The purpose of the present study was therefore to investigate the effect of central and peripheral 5-HT on glucose homeostasis in the rainbow trout.

MATERIALS AND METHODS

Animals

Immature rainbow trout (*Oncorhynchus mykiss*; 100±40 g) were obtained from a commercial trout farm (Soutorredondo, Noia, Spain). Fish were acclimated in 200 litre tanks for 3 weeks in running dechlorinated tap water (temperature 14±0.5°C, pH 6.3±0.1) with continuous aeration, and maintained under a 12 h:12 h light:dark photoperiod (lights on at 8:00 h). Fish were fed once daily, in the morning (at 12:00 h), with commercial dry pellets (ration equivalent to 1.5% of body mass per day), and were not fed in the 24 h prior to experiments. All experiments were carried out in the morning to avoid possible effects of circadian variations. Fish were anaesthetised before handling, injection or sampling, with 50 mg l^{-1} tricaine methanesulphonate (MS-222; Sigma, Sigma Chemical Co., St Louis, USA) buffered to pH 7.4 with sodium bicarbonate. The anaesthetic was added directly to the tank where the fish were held, as this type of administration efficiently reduces the release of catecholamines induced by handling stress in the rainbow trout (Caamaño-Tubio et al., 2010). Replicate (triplicate or more) tanks were established for each experiment.

Drugs used and drug administration

Serotonin (5-hydroxytryptamine creatinine sulphate), d-fenfluramine (d-FF; an indirect serotonergic agonist) and WAY 100635 maleate (WAY; a 5-HT1A receptor antagonist), were purchased from Sigma Chemical Co (St Louis, USA). Mianserin hydrochloride (MIAN; a 5-HT2 receptor antagonist), 5-carboxamidotryptamine maleate (5-CT; a 5-HT1 receptor agonist), 1-(3-trifluromethyphenyl) piperazine hydrochloride (TFMPP; a 5-HT1B receptor agonist), pimozide (PIM; a 5-HT7 receptor antagonist), α-methyl-5-hydroxytryptamine maleate (α-m-5-HT; a 5-HT2 receptor agonist), BRL 15572 hydrochloride (BRL; a 5-HT1D receptor antagonist) and SB 204741 (SB; a 5-HT2B receptor antagonist), were obtained from Tocris Cookson Ltd (Bristol, UK).

Drugs (5-HT and 5-HT agonists and antagonists) were administered by intraperitoneal (i.p.) injection in 125 µl of saline (SAL; 0.6% w/v NaCl), except for PIM, BRL and SB, which were dissolved in a vehicle (VEH) of propylenglycol and dimethylsuphoxide (PPG and DMSO) in proportions of 1:6, 1:9 and 1:2, respectively. The intracerebroventricular (i.c.v.) administration of 5-HT (in 2 µl of saline per fish, 0.6% w/v NaCl) was as previously described (Aldegunde and Mancebo, 2006). Briefly, the i.c.v. administration was carried out with a 10 µl Hamilton microsyringe. On the day of the experiment, the needle was introduced into the third ventricle at the level of the preoptic nuclei, just at the level of the anterior point of union of the lobes of the optic tectum; the microsyringe was held in place with a micromanipulator while the 2 µl dose was infused.

Effect of intraperitoneal and intracerebroventricular administration of 5-HT on plasma glucose and catecholamine levels: dose and time response

One series of experiments (dose–response experiments) was designed to investigate the effect of the i.p. administration of different doses of 5-HT (0.5, 1, 2, 5 and 10 mg kg^{-1}) 150 min after their administration (N=8 fish per dose). A second series of experiments (time–response experiments) examined the effect of the administration of a dose of 5-HT (2 mg kg^{-1}), with samples taken at 0, 50, 100, 150 and 200 min after administration (N=6–7 fish each time). The plasma levels of adrenaline (A) and noradrenaline (NA) were also determined at 0, 100, 150 and 200 min after administration of a dose of 5-HT (2 mg kg^{-1}; N=4–6). In all experiments samples of blood were taken for later determination of glucose or catecholamine levels.

To study the effect of i.c.v. administration of 5-HT on plasma glucose and hepatic glycogen levels, a series of dose–response experiments was carried out with the following doses of 5-HT (5.12, 12.8, 32, 80 and 200 µg kg^{-1}). Blood samples were extracted from the fish 60 min after administration of 5-HT, then the fish were decapitated and a sample of liver taken from each (N=10 fish per dose). A second series, of time–response experiments, examined the effect of the i.c.v. administration of a dose of 5-HT (12.8 µg kg^{-1}), and in this case the samples (blood and liver) were taken 20, 60, 120 and 180 min after administration (N=6–10 fish per group and time).

5-HT and the blood-brain barrier

This experiment was carried out specifically to determine the capacity of the blood-brain barrier (BBB) to block the passage of peripherally administered 5-HT to the brain. For this purpose, we used [^{14}C]5-HT (56 mCi mmol l^{-1}; Amersham Biosciences UK Limited, UK). The final concentration of 5-HT in the working solution (non-radioactive 5-HT + [^{14}C]5-HT) was 6.36 mmol l^{-1}, which is equivalent to i.p. administration of 2 mg kg^{-1} in a volume of 125 µl per fish. Samples of blood and brain were taken 15 min after the i.p. administration of [^{14}C]5-HT. The radioactive content was determined, 5 min per sample, in a liquid scintillation counter (LS-6500, Beckman, USA), and the number of counts per minute detected in the plasma and in the brain tissue were expressed in nanomoles of 5-HT, based on the counts in the working solution of known concentration (N=8). Times in the order of 10–15 min are commonly used in studies of this type in mammals (Buschiazzo et al., 1970; Vitte et al., 1988; Naylor et al., 1995; Preston et al., 1995; Perasso et al., 2003) and fish (Aldegunde et al., 2000). If a substance passes the BBB it will actually be detectable in the brain long before 15 min. At the end of the study the fish and associated material were disposed of appropriately for radioactive material.

Effect of intraperitoneal administration of pharmacological agents

The effect of two different doses of fenfluramine (5 and 15 mg kg^{-1}) were investigated, 150 min after i.p. administration (N=6–8 fish per group and dose). In both cases, blood samples were taken for determination of glucose levels. The plasma levels of catecholamines were also determined in fish administered the lowest dose of d-FF (N=7 fish per group and dose).

To study the effect of the 5-HT2 receptor antagonist (MIAN), four different groups were established: (1) SAL–SAL, (2) SAL–MIAN (5 mg kg^{-1}), (3) SAL–5-HT (2 mg kg^{-1}) and (4) MIAN (5 mg kg^{-1})–5-HT (2 mg kg^{-1}; N=6–7 fish per group). In all experiments in which antagonists were administered, the time between the first (antagonist) and second (5-HT) injections was 30 min. Blood samples were taken, for later determination of glucose and/or of catecholamines, 150 min after the second i.p. injection.

In addition, to study the effect of the receptor agonists for 5-HT1 (5-CT), 5-HT1B (TFMPP) and 5HT2 (α-m-5-HT), two different experiments were carried out. In the first (N=6 fish per group) three

groups were established: (1) SAL, (2) 5-CT (1 mg kg^{-1}) and (3) TFMPP (2 mg kg^{-1}). In the second experiment (N=8 fish per group), two groups were established: (1) SAL and (2) α-m-5-HT (5 mg kg^{-1}). Blood samples were always collected 150 min after i.p. administration. In the first experiment, blood samples were taken for later determination of glucose and catecholamine levels, whereas in the second group, only the glucose levels were determined.

To study the effect of the receptor antagonists for 5-HT1A (WAY), 5-HT1D (BRL), 5-HT2B (SB) and 5-HT7 (PIM), the same timing was used for the injection and collection of blood samples for glucose analysis, as described at the beginning of this section. For the 5-HT1A antagonist, three treatments were considered: (1) SAL–SAL, (2) SAL–5-HT (2 mg kg^{-1}) and (3) WAY (2 mg kg^{-1})–5-HT (2 mg kg^{-1}; N=8 fish per group). The experiment was repeated with the following modifications: 5-HT and WAY were injected consecutively, one immediately after the other, and the blood samples were collected after 75 min. The dose of WAY was 2.5 mg kg^{-1} (N=12–16). For the other antagonists, different groupings were established, as follows; for 5-HT1D: (1) VEH–SAL, (2) VEH–5-HT (2 mg kg^{-1}) and (3) BRL (5 mg kg^{-1})–5-HT (2 mg kg^{-1}); for the 5-HT2B antagonist: (1) VEH–SAL, (2) VEH–5-HT (2 mg kg^{-1}) and (3) SB (2 mg kg^{-1})–5-HT (2 mg kg^{-1}); and for the 5-HT7antagonist: (1) VEH–SAL, (2) VEH–5-HT (2 mg kg^{-1}) and (3) PIM (10 mg kg^{-1})–5-HT (2 mg kg^{-1}). The number of fish per group was 8–12.

Finally, to study the combined effect of the antagonists of 5-HT2B (SB) and 5-HT1A (WAY), three groups were considered: (1) VEH/SAL–SAL (2) VEH/SAL–5-HT (2 mg kg^{-1}), and (3) SB (2 mg kg^{-1})/WAY (2 mg kg^{-1})–5-HT (2 mg kg^{-1}). The VEH/SAL and the antagonists were injected consecutively, one immediately after the other, and 5-HT was injected i.p. 30 min later. Blood samples were collected, for determination of glucose levels, 150 min after the second injection (N=11–12 fish per group).

As most of the drugs were being used for the first time in fish (rainbow trout), we based the doses on those used in mammals. We decided to use doses within the medium to high range of those used in mammals (Niesink and Van Ree, 1982; Scotti de Carolis et al., 1986; Schechter, 1988; Hagan et al., 1997; Guscott et al., 2003; Morita et al., 2005; Freitas et al., 2006; Egashira et al., 2008).

Tissue sampling and analytical procedures

Blood was obtained by caudal puncture with ammonium-heparinized syringes (Sigma, Sigma Chemical Co., St Louis, USA), and plasma was obtained after centrifugation of the blood (11,600 ***g*** for 5 min at 2–3°C). Aliquots of plasma (250 µl) for posterior catecholamine and glucose quantification, and tissue liver samples, for glycogen quantification, were stored at –80°C until their respective analysis. Liver samples (200 mg) were placed on ice and homogenized at 12,500 r.p.m. for 15 s, with ice-cooled 6 mol l^{-1} perchloric acid ($HClO_4$), neutralized (with 1 mol l^{-1} $NaHCO_3$), centrifuged (2 min at 9,000 ***g***), and the supernatant stored at –80°C until glycogen assay (Figueroa et al., 2000). Glucose obtained after glycogen breakdown, and plasma glucose levels were determined by the glucose-oxidase–peroxidase method (SPINREACT, S.A., Girona, España). Plasma A and NA levels were determined on alumina (aluminium oxide)-extracted samples, by high-pressure liquid chromatography with electrochemical detection (HPLC-EC) (Caamaño-Tubio et al., 2010). Briefly, aliquots of plasma (250 µl) were mixed with DHBA (3,4-dihydroxybenzylamine hydrobromide, Sigma Chemical Co., St Louis, USA), added as an internal standard (40 µl of 100 ng ml^{-1} of an aqueous solution of 0.1 mol l^{-1} $HClO_4$), and 125 µl of 0.4 mol l^{-1} $HClO_4$. After stirring for 1 min, samples were centrifuged for 5 min (11,600 ***g***, 2–3°C). The supernatant was then transferred to a tube containing 50 mg acid alumina (Bio-Rad Laboratories, Richmond, California, USA), 250 µl of Tris-EDTA buffer 1.5 mol l^{-1} (pH 8.6) and 100 µl of EDTA 0.8 mol l^{-1}. The tubes were then vortexed for 10 min during which time the catecholamines were adsorbed onto the alumina. This was followed by centrifugation at 11,600 ***g*** and subsequent washing of the alumina three times with 1 ml of chilled ultrapure water. For elution of catecholamines, 100 µl of 0.1 mol l^{-1} $HClO_4$ was added to the alumina and stirred for 3 min. After centrifugation for 5 min (11,600 ***g***), aliquots of the supernatant were stored at –80°C until A and NA analysis by HPLC-EC. Briefly, the chromatographic system consisted of a Waters M510 solvent delivery pump, a Supelcosil LC-18-DB reverse-phase analytical column (5 µm particle size, 150 mm×4.6 mm), and a Coulochem M5100 A detector, which included an analytical cell set at –0.50 mV (first electrode) and +330 mV (second electrode). The mobile phase, composed of a mixture of sodium acetate (93.3 mmol l^{-1}), citric acid (11.34 mmol l^{-1}), octanesulphonic acid (70 mg l^{-1}) and Na_2-EDTA (10 mg l^{-1}; final pH 5.25), was pumped isocratically at a flow rate of 1.5 ml min^{-1} at room temperature. The concentrations of A and NA were estimated from data obtained from sample aliquots (20 µl) injected into the chromatographic system. Adrenaline (hydrated epinephrine tartrate) and noradrenaline (hydrated norepinephrine tartrate) were purchased from Sigma Chemical Co., St Louis, USA).

Statistical analysis

All data are presented as means ± standard error of the mean (s.e.m.). Statistical significance was evaluated with Student's t-test for comparison between two groups. In all other cases, one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparison test were used. All analyses were carried out with commercial software (SigmaStat v2.0, SPSS Scientific Inc.).

RESULTS

In fish given i.p. injections of different doses of 5-HT (0.5, 1, 2, 5 and 10 mg kg^{-1}) there was a dose-dependent (P<0.001) increase in plasma glucose levels 150 min after administration of the compound (Fig. 1A). There was also a time-dependent (P<0.001) increase in plasma glucose levels 0, 50, 100, 150 and 200 min after 5-HT administration (2 mg kg^{-1}; Fig. 2B). In fish injected with saline solution no significant variations in plasma levels of glucose were observed. In fish administered 5-HT (2 mg kg^{-1}), there were significant increases (P<0.05) in plasma levels of A and NA, although the levels of NA were only significant after 150 and 200 min (Fig. 1C,D).

In fish given i.c.v. injections of different doses of 5-HT (5.12, 12.8, 32, 80 and 200 µg kg^{-1}) there were no significant variations in plasma glucose (Fig. 2A) or liver glycogen (Fig. 2C) levels 60 min after administration. Likewise, no significant variations in plasma glucose levels were observed 20, 60, 120 and 180 min after i.c.v. administration of one dose of 5-HT (12.8 µg kg^{-1}; Fig. 2B), or in liver glycogen (Fig. 2D).

The i.p. administration of two different doses of d-FF (5 mg kg^{-1} and 15 mg kg^{-1}), a drug capable of inducing 5-HT release, led to a significant (P<0.05) dose-dependent increase in plasma levels of glucose (Fig. 3A). Intraperitoneal administration of d-FF (5 mg kg^{-1}) induced increases in A and NA levels, which was only statistically significant (P<0.05) in the case of A (Fig. 3B).

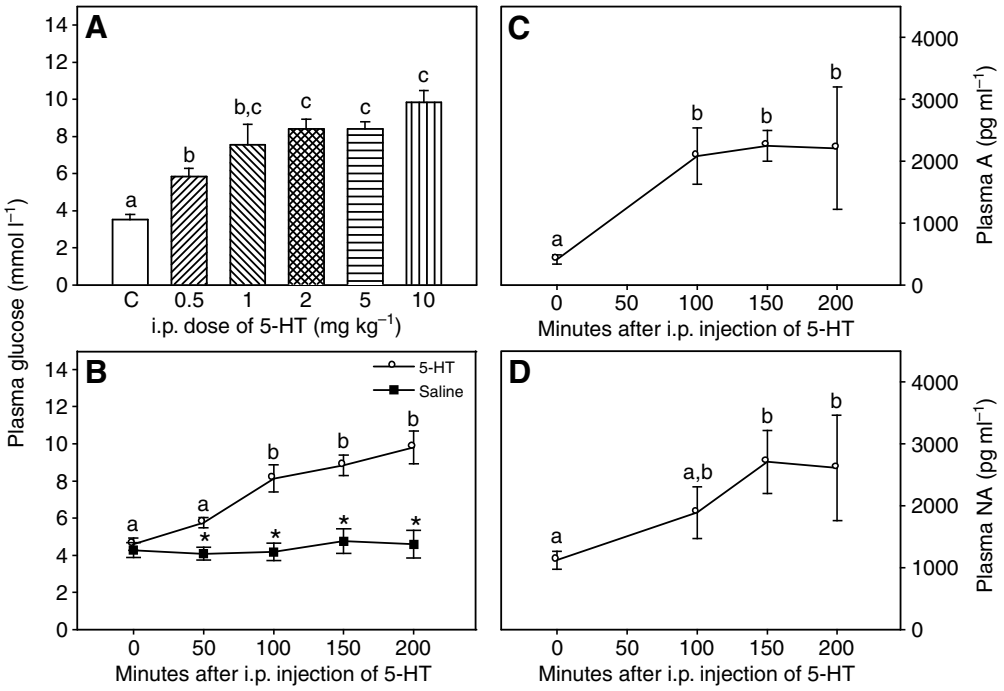

Fig. 1. Effects of i.p. administration of 5-HT (serotonin) on plasma glucose and catecholamine levels. (A) Dose-dependent increase in plasma levels of glucose 150 min after i.p. injection of 5-HT (N=8). (B) Effects of i.p. injection of 5-HT (2 mg kg^{-1}) on plasma levels of glucose at different times (N=6–7). (C,D) Effects of i.p. injection of 5-HT (2 mg kg^{-1}) on plasma levels of adrenaline (A; C) and noradrenaline (NA; D) at different times (N=4–6). Values are means ± s.e.m. (N, number of trout in each group). Different letters indicate significant differences between samples (P<0.05). *Significant differences between treatments (P<0.05).

After i.p. administration of [^{14}C]5-HT, 0% radioactivity was detected in the brain (data not shown). The radioactivity of the solution of [^{14}C]5-HT injected into the fish, should be more than enough to detect any 5-HT in the brain if present.

The i.p. administration of 5-HT (SAL–5-HT) and MIAN–5-HT produced a significant level of hyperglycaemia (P<0.05) relative to the control (MIAN–SAL; Fig. 4A). The increases in glucose levels caused by these two treatments were not significantly different from each other. The treatments (SAL–5-HT or MIAN–5-HT) resulted in significant increases (P<0.05) in the levels of A (Fig. 4B) relative to the control (MIAN–SAL). Administration of 5-HT (SAL–5-HT) led to a significant increase (P<0.05) in the levels of NA, but administration of MIAN–5-HT, did not result in significantly different levels of NA (Fig. 4C) relative to the control (MIAN–SAL). No significant differences between the MIAN–SAL group (used as a control) and the SAL–SAL group were observed in any of the cases (data not shown).

Administration of 5-CT, but not TFMPP, induced a significant hyperglycaemic effect (P<0.05) (Fig. 5A). Administration of 5-CT induced a significant increase (P<0.05) in plasma A compared with

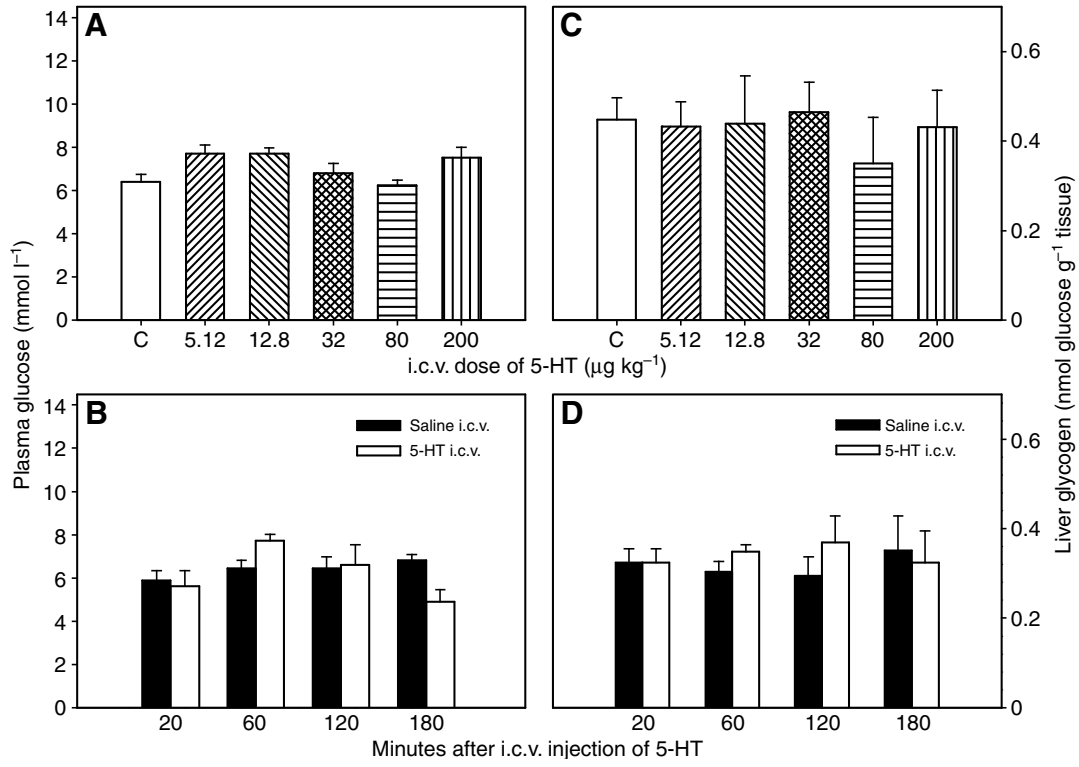

Fig. 2. Effects of i.c.v. administration of 5-HT (serotonin) on plasma glucose and hepatic glycogen. (A) Plasma levels of glucose 60 min after i.c.v. injection of different doses of 5-HT (N=10). (B) Plasma levels of glucose at different times after i.c.v. injection of 5-HT (12.8 μg kg^{-1}; N=6–10). (C) Hepatic levels of glycogen 60 min after i.c.v. injection of different doses of 5-HT (N=10). (D) Effects of i.c.v. injection of 5-HT (12.8 μg kg^{-1}) on hepatic glycogen at different times (N=6–10). Values are means ± s.e.m. (N, number of trout in each group). There were no significant differences (P<0.05).

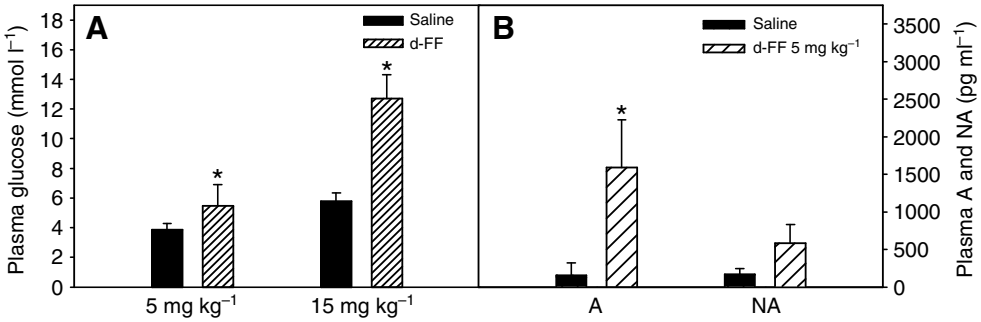

Fig. 3. Effects of d-fenfluramine (d-FF) on plasma glucose and catecholamine levels. (A) Plasma levels of glucose 150 min after i.p. injection of d-FF (5 and 15 mg kg^{-1}; N=6–8). (B) Plasma levels of adrenaline (A) and noradrenaline (NA) 150 min after i.p. injection of d-FF (5 mg kg^{-1}; N=6–8). Values are means ± s.e.m. (N, number of trout in each group; *P<0.05).

the control (Fig. 5B). The TFMPP treatment did not significantly alter the levels of A (Fig. 5B). Neither 5-CT nor TFMPP significantly altered plasma levels of NA (Fig. 5C). Treatment with α-m-5-HT (5-HT2 receptor agonist) induced a slight, non significant increase in plasma levels of glucose (data not shown).

Analysis of the effect of the pretreatment with the antagonist 5-HT1A revealed that the i.p. administration of 5-HT (VEH–5-HT) and WAY-5-HT induced a significant level of hyperglycaemia (P<0.05) relative to the control (VEH–SAL). The increases in the levels of glucose caused by these two treatments were not significantly different (Fig. 6A). However, under the modified conditions, there appeared to a tendency for WAY to counteract the

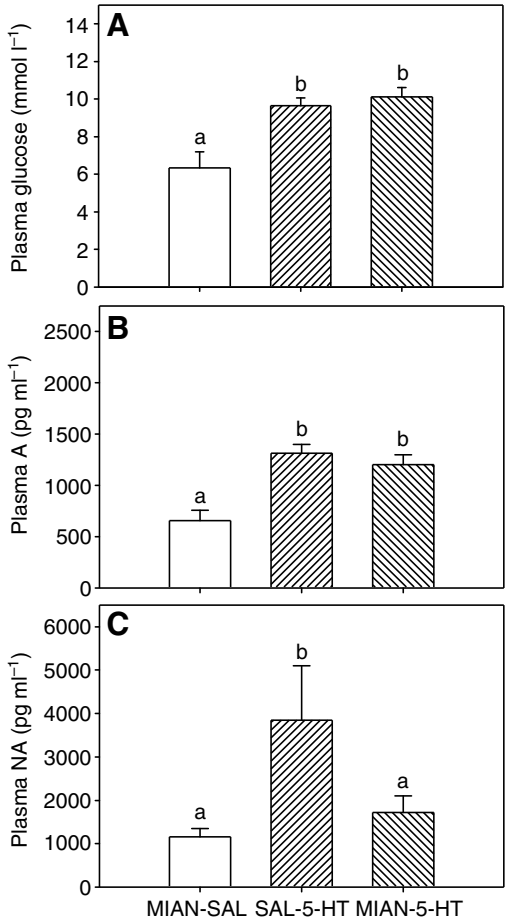

Fig. 4. Effect of mianserin (MIAN; a 5-HT2 antagonist) on the hyperglycaemic effect of 5-HT and on catecholamine levels. Effect of pretreatment with MIAN on: (A) Plasma levels of glucose (N=6–7), (B) plasma levels of adrenaline (A; N=6–7) and (C) plasma levels of noradrenaline (NA; N=6–7). MIAN (5 mg kg^{-1}) was administered intraperitoneally 30 min before 5-HT (2 mg kg^{-1}) and the parameters were measured 150 min after administration of 5-HT. Values are means ± s.e.m. (N, number of trout in each group). Different letters over the bars indicate significant differences (P<0.05). SAL, saline.

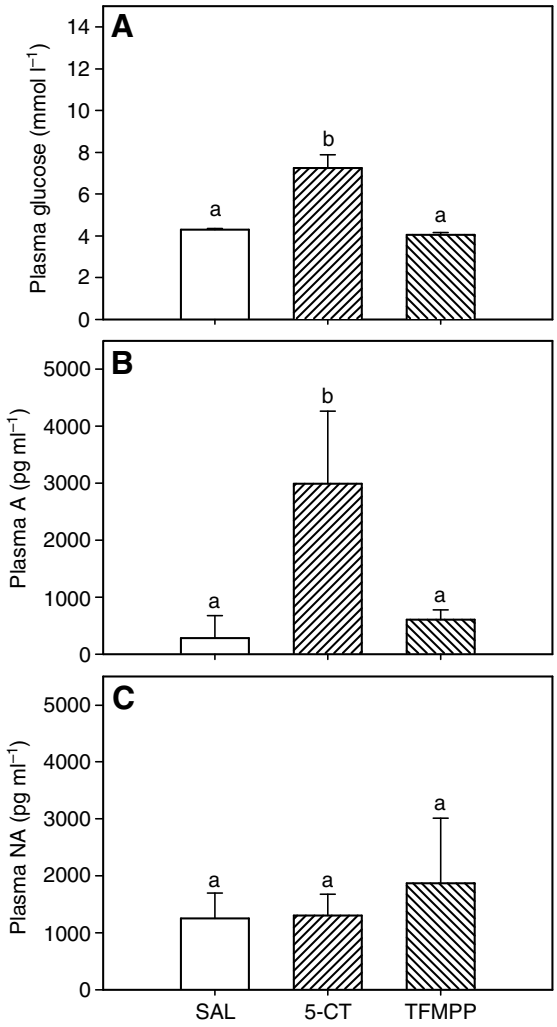

Fig. 5. Effects of 5-carboxamidotryptamine (5-CT; a 5-HT1 agonist) and 3-trifluoromethylphenylpiperazine (TFMPP; a 5-HT1B agonist) on plasma glucose and catecholamine levels. (A) Effects of 5-CT (1 mg kg^{-1}) and TFMPP (2 mg kg^{-1}), 150 min after i.p. administration, on plasma levels of glucose (N=6). (B,C) Effects of 5-CT and TFMPP, 150 min after i.p. administration, on plasma levels of adrenaline (A; N=6) and noradrenaline (NA; N=6), respectively. Values are means ± s.e.m. (N, number of trout in each group). Different letters over the bars indicate significant differences (P<0.05). SAL, saline.

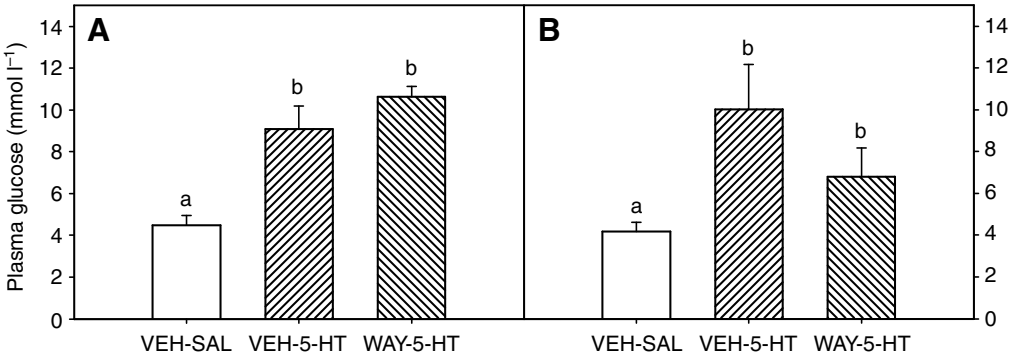

Fig. 6. Effects of WAY 100635 maleate (WAY; a 5-HT1A antagonist) on the hyperglycaemic effect of 5-HT. (A) Plasma levels of glucose after pretreatment with WAY (2 mg kg^{-1}). This antagonist was administered (i.p.) 30 min before 5-HT, and glucose was analysed 150 min after its administration (N=8). (B) Plasma levels of glucose after administration of WAY (2.5 mg kg^{-1}). This antagonist was administered at the same time as 5-HT, and glucose levels determined 75 min after the administration (N=12). Values are means ± s.e.m. (N, number of trout in each group). Different letters over the bars indicate significant differences (P<0.05). VEH, vehicle; SAL, saline; 5-HT, serotonin.

hyperglycaemic effect of 5-HT (Fig. 6B). Following i.p. administration of the antagonists of 5-HT1D (BRL), 5-HT2B (SB) and 5-HT7 (PIM) receptors, in all cases the levels of glucose in the groups subjected to the treatment (VEH–5-HT and 5-HT–antagonist) were higher than in the respective controls, but there were no significant differences between treated groups in any cases (Fig. 7A–C).

Finally, the administration of 5-HT (VEH/SAL–5-HT) and of the combined treatment with SB/WAY-5-HT induced a significant hyperglycaemic effect (P<0.05). However, there were no significant differences between the levels of glucose in fish administered VEH/SAL–5-HT and those administered SB/WAY-5-HT (Fig. 8).

DISCUSSION

The above results demonstrate, for the first time in a teleost, that peripheral (but not central) 5-HT exerts a hyperglycaemic effect. This effect is mediated by activation of 5-HT1-like receptors and release of adrenaline.

The first set of experiments investigated the possible modulatory role of 5-HT on blood glucose. The results demonstrate that the i.p. administration of 5-HT induces a clear hyperglycaemia, similar to that observed in mammals after i.p. administration of similar doses of 5-HT (Yamada et al., 1995). Given that in mammals the hyperglycaemic effect of 5-HT may be mediated by its action on the central and/or peripheral system, we investigated whether this was also true in fish. For this purpose, we first tested whether the peripherally administered 5-HT is capable of passing the BBB. As no radioactivity was detected in the brain 15 min after the i.p. administration of [^{14}C]5-HT, we deduced that 5-HT does not pass the BBB, or that it only does so in very small quantities. This is consistent with findings in mammals (Axelrod and Inscoe, 1963; Davson and Segal, 1996) and with the fact that in most studies carried out on teleosts, the BBB has been found to be effective, although to different degrees (Lundquist, 1942; Davson and Segal, 1996). Further evidence for the lack of transfer of peripheral 5-HT to the brain is that although central administration of 5-HT has a strong anorexigenic effect, peripheral administration (i.p.) does not have any effect on feeding in the goldfish (De Pedro et al., 1998). By contrast, i.p. administration of d-FF, an indirect 5-HT agonist that passes the BBB, is capable of inducing a strong anorexigenic action (Ruibal et al., 2002). As 5-HT does not pass the BBB, we suggest that in the rainbow trout, the hyperglycaemic effect resulting from the i.p. administration of 5-HT is mediated by its action on the peripheral system. However, it is important to determine whether or not the hyperglycaemic action is a specific physiological response, i.e. if the release of endogenous 5-HT from its peripheral deposits is capable of replicating the action. The results do not leave any room for doubt: the i.p. administration of d-FF, a drug capable of inducing the release of 5-HT in the CNS (Ruibal et al., 2002) and from the peripheral stores of 5-HT in the rainbow trout (Caamaño-Tubio et al., 2007), mimics the hyperglycaemic action of 5-HT in a dose-dependent fashion. At the moment it is not possible to determine the specific origin of the stores of 5-HT involved.

The fact that 5-HT exerts a peripherally mediated hyperglycaemic effect does not exclude the possibility that it may also exert a regulatory effect on blood glucose by acting centrally. However, in light of the lack of alterations in the levels of hepatic glycogen (an index of the release of catecholamines) and of plasma glucose after the i.c.v. administration of 5-HT, this possibility can be discounted. The results are not consistent with those observed in mammals and show that in the rainbow trout, cerebral 5-HT does not act as a neuroregulator of the sympathetic–chromaffin cell nervous system. In relation to the above, we have previously observed that the cerebral hypoglycaemia induced by i.c.v. administration of 2-deoxyglucose is incapable of stimulating the sympathetic nervous system and inducing an increase in plasma glucose levels in rainbow trout (Soengas and Aldegunde, 2004), in contrast with the observations made in mammals (Muller et al., 1971; Weidenfeld et al., 1994). The available data indicate differences in the central mechanisms for regulating blood glucose levels in mammals and in rainbow trout.

It is known that in the rainbow trout, 5-HT may induce the release of catecholamines (Fritsche et al., 1993). Indeed, in the present study, i.p. administration of 5-HT induced an increase in levels of A and NA, and moreover the effect was partially mimicked by the endogenous 5-HT released by the action of d-FF. In both cases, the increases in plasma catecholamines were associated with an increased in blood glucose. The possible catecholaminergic mediation of the hyperglycaemic effect of the 5-HT is consistent with findings in mammals, in which: (1) the hyperglycaemic effect of 5-HT, whether *via* its central or peripheral action, may be mediated by the release of A from the adrenal medulla (Yamada et al., 1995; Sugimoto et al., 1996b; Chaouloff et al., 1990b), and (2) d-FF produces hyperglycaemia, mediated by the release of adrenal catecholamines (Chaouloff et al., 1992). However, interestingly, the increase in levels of 5-HT within physiological limits (treatment with d-FF) induces,

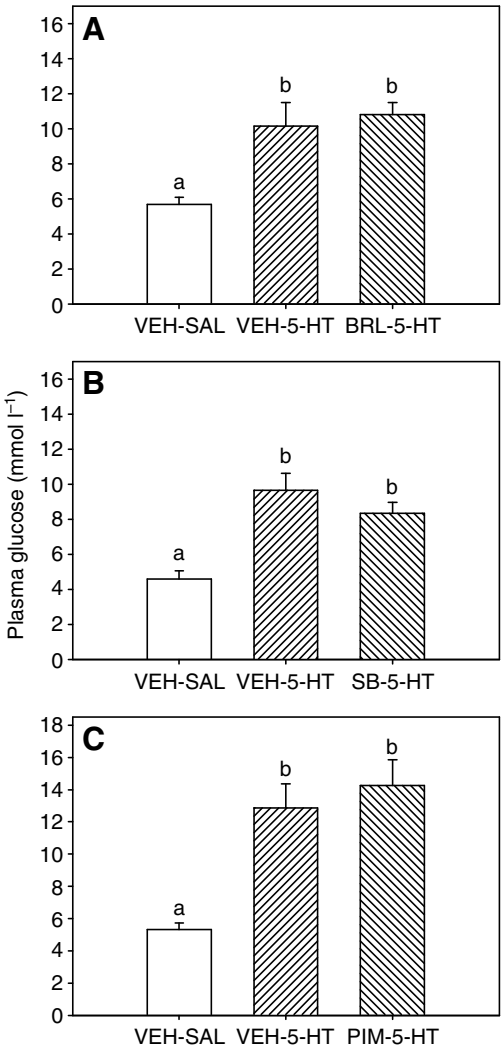

Fig. 7. Effects of BRL 15572 hydrochloride (BRL; a 5-HT1D antagonist), SB 204741 (SB; a 5-HT2B antagonist) and pimozide (PIM; a 5-HT7 antagonist) on the hyperglycaemic effect of 5-HT. (A–C) Plasma levels of glucose after pretreatment with (A) BRL (5 mg kg^{-1}; *N*=9–10), (B) SB (2 mg kg^{-1}; *N*=12) or (C) PIM (10 mg kg^{-1}; *N*=9–12). In all cases the antagonist was administered (i.p.) 30 min before the 5-HT (2 mg kg^{-1}) and the glucose analyzed 150 min after its administration. Values are means ± s.e.m. (*N*, number of trout in each group). Different letters over the bars indicate significant differences (P<0.05). VEH, vehicle; SAL, saline; 5-HT, serotonin.

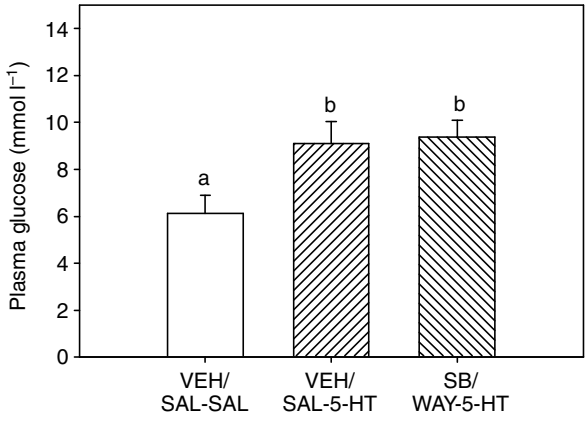

Fig. 8. Effects of the combined administration of SB 204741 (SB; a 5-HT2B antagonist) and WAY 100635 maleate (WAY; a 5-HT1A antagonist) on the hyperglycaemic effect of 5-HT. Glucose levels after pretreatment with SB (2 mg kg^{-1}) and WAY (2 mg kg^{-1}). Both antagonists were administered (i.p.) at the same time, 30 min before 5-HT (2 mg kg^{-1}) and glucose levels measured 150 min after their administration (*N*=11–12). Values are means ± s.e.m. (*N*, number of trout in each group). Different letters over the bars indicated significant differences (P<0.05). VEH, vehicle; SAL, saline; 5-HT, serotonin.

in addition to a hyperglycaemic effect, differential action on the levels of A and NA, with a clearly predominant effect on A. The effect of d-FF on A but not NA lends support to the idea that A and not NA stimulates glucose release. The possible differential regulation of the release of A and NA is totally consistent with the fact that catecholamines are stored in different populations of chromaffin cells in fish (Gallo and Civinini, 2003) and that regulation of their release is also different in these animals (Reid et al., 1994; Bernier and Perry, 1996; Al-Kharrat et al., 1997).

Considering that in mammals the 5-HT2 and 5-HT1A receptors are closely involved in the hyperglycaemic effect of 5-HT (Sugimoto et al., 1996b; Chaouloff et al., 1990b), we attempted to determine possible mediation of this effect in the rainbow trout. This investigation was initiated by studying the effect of MIAN, a generic antagonist of 5-HT2 receptors, on the hyperglycaemic action of 5-HT. The results show that this antagonist was not capable of blocking the hyperglycaemic action of 5-HT and of the release of A, but that it was capable of blocking the release of NA. Interpretation of these results leads us to three important conclusions: (1) that the hyperglycaemic action of 5-HT must be mediated mainly by A, (2) that the 5-HT2-type receptors may mediate the release of NA but not of A, and (3) that the 5-HT2 type receptors do not appear to play an important role in mediating the 5-HT-dependent hyperglycaemia. The relative unimportance of the 5-HT2-type receptors in mediating the effect is confirmed by the fact that neither the administration of α-m-5-HT (5-HT2 agonist) or of SB (selective 5-HT2B antagonist) was capable of increasing blood glucose levels or of blocking the hyperglycaemic action of 5-HT, respectively. By contrast, when 5-CT (a non selective 5-HT1 agonist) was administered, the results were conclusive. Administration of 5-CT increased the levels of both glucose and of A, but not of NA. It appears evident that in the rainbow trout, the hyperglycaemic action of 5-HT is mediated by activation of 5-HT1-type receptors and the subsequent release of adrenaline. Obviously, in addition to the role of A, we cannot rule out a possible role for 5-HT (*via* 5-HT receptors expressed in the interrenal cells) in increasing the secretion of cortisol, which would result in an increase in glucose. It would be interesting to explore this possibility in future studies.

Based on the fact that in mammals 5-CT shows affinity for 5-HT1B, 5-HT1D, 5-ht5A, 5-HT7 and particularly, subtype 5-HT1A receptors (Yamada et al., 1998), we used a series of agonists and antagonists in an attempt to determine the subtype of 5-HT receptor involved. Under the experimental conditions of the present study, there was an absence of any role of the 5-HT1A, 5-HT1B, 5-HT1D and 5-HT7 receptors in the hyperglycaemic action of 5-HT. This is not consistent with the findings for 5-HT1A (Chaouloff et al., 1990c; Chaouloff et al., 1990a; Uvnäs-Moberg et al., 1996; Sugimoto et al., 2001) and 5-HT7 (Yamada et al., 1998), although it is consistent with the findings for 5-HT1B (Chaouloff et al., 1990b; Uvnäs-Moberg et al., 1996) in relation to mammals. The role of the 5-ht5A receptor was not evaluated in the present study, and therefore the possibility that it may at least partly mediate the hyperglycaemic action of 5-HT cannot

be ruled out. Nevertheless, taking into account previously reported data in mammals (Hannon and Hoyer, 2008; Jonnakuty and Gragnoli, 2008), it can be concluded that this receptor is highly unlikely to play any role in the hyperglycaemic action of 5-HT.

The results demonstrate that in the rainbow trout, peripheral (but not central) 5-HT modulates homeostasis of peripheral glucose, with a hyperglycaemic effect. This effect is associated with the release of A and activation of 5-HT1-like receptors, which as far as could be ascertained in the present study, do not possess the pharmacological characteristics of subtypes 5-HT1A, 5-HT1B and 5-HT1D of mammals. The subtypes of 5-HT receptors in the fish nervous system have not yet been fully defined (Wikstrom et al., 1995; Winberg and Nilsson, 1996; Agrawal and Omeljaniuk, 2000) and even less is known about them in the peripheral system and in non-neuronal tissue, although subtypes that are clearly different from those found in mammals have been reported (Bakker et al., 1993; Janvier et al., 1996; Cerda et al., 1997; Sundin et al., 1998). Finally, the 5-HT2 type receptors that may mediate the release of NA, do not appear to play an important role in the hyperglycaemic effect induced by 5-HT.

LIST OF SYMBOLS AND ABBREVIATIONS

A	adrenaline
BRL	BRL 15572 hydrochloride
5-CT	5-carboxamidotryptamine maleate
d-FF	d-fenfluramine
DMSO	dimethylsulphoxide
HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptamine or serotonin
α-m-5-HT	α-methyl-5-hydroxytryptamine maleate
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
MIAN	mianserin hydrochloride
NA	noradrenaline
PIM	pimozide
PPG	propylenglycol
SAL	saline
SB	SB 204741
TFMPP	1-(3-trifluromethyphenyl)piperazine hydrochloride
VEH	vehicle
WAY	WAY 100635 maleate

ACKNOWLEDGEMENTS

This study was supported by a Research Grant from the Xunta de Galicia (PGIDIT03PXIB20001PR and INCITE08PXIB2000053PR) to M.A. J.P.M. was the recipient of a predoctoral fellowship from Xunta de Galicia (programa Maria Barbeito). The experiments described comply with the principles of animal care established by the Spanish government and the European Union (L358/1, 18/12/1986).

REFERENCES

Agrawal, S. M. and Omeljaniuk, R. J. (2000). Specific binding of [^{3}H]ketanserin to hypothalamus membranes of juvenile rainbow trout, *Oncorhynchus mykiss*. *Can. J. Physiol. Pharmacol.* **78**, 58-66.

Aldegunde, M. and Mancebo, M. (2006). Effects of neuropeptide Y on food intake and brain biogenic amines in the rainbow trout (*Oncorhynchus mykiss*). *Peptides* **27**, 719-727.

Aldegunde, M., Andrés, M. D. and Soengas, J. L. (2000). Uptake of 3-O-methyl-D-[U-14]glucose into brain of rainbow trout: possible effects of melatonin. *J. Comp. Physiol. B* **170**, 237-243.

Al-Kharrat, H., Weiss, U., Tran, Q., Nibbio, B., Scholz, S. and Epple, A. (1997). Cholinergic control of catecholamine release in the eel. *Gen. Comp. Endocrinol.* **108**, 102-108.

Axelrod, J. and Inscoe, J. K. (1963). The uptake and binding of circulating serotonin and the effect of drugs. *J. Pharmacol. Exp. Ther.* **141**, 161-165.

Bakker, R., Dekker, K., De Jonge, H. R. and Groot, J. A. (1993). VIP, serotonin, and epinephrine modulate the ion selectivity of tight junctions of goldfish intestine**.** *Am. J. Physiol.* **264**, R362-R368.

Bernier, N. J. and Perry, S. F. (1996). Control of catecholamine and serotonin release from the chromaffin tissue of the Atlantic hagfish. *J. Exp. Biol.* **199**, 2485-2497.

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M. and Blanchard, R. J. (1993). Subordination stress: Behavioural, brain and neuroendocrine correlates. *Behav. Brain Res.* **58**, 113-121.

Buschiazzo, P. M., Terrell, E. B. and Regen, D. M. (1970). Sugar transport across the blood-brain barrier. *Am. J. Physiol.* **219**, 1505-1513.

Caamaño-Tubio, R., Perez, J., Ferreiro, S. and Aldegunde, M. (2007). Peripheral serotonin dynamics in the rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* **145C**, 245-255.

Caamaño-Tubio, R., Weber, R. A. and Aldegunde, M. (2010). Home tank anesthesia: a very efficient method of attenuating handling stress in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *J. Appl. Ichthyol.* **26**, 116-117.

Cerda, J., Petrino, T. R., Greenberg, M. J. and Wallace, R. A. (1997). Pharmacology of the serotonergic inhibition of steroid-induced reinitiation of oocyte meiosis in the teleost *Fundulus heteroclitus*. *Mol. Reprod. Dev.* **48**, 282-291.

Chaouloff, F. and Jeanrenaud, B. (1987). 5-HT1A and alpha-2 adrenergic receptors mediate the hyperglycemic and hypoinsulinemic effects of 8-hydroxy-2-(di-n-propylamino)tetralin in the conscious rat. *J. Pharmacol. Exp. Ther.* **243**, 1159-1166.

Chaouloff, F., Baudrie, V. and Laude, D. (1990a). Evidence that 5-HT1A receptors are involved in the adrenaline-releasing effects of 8-OH-DPAT in the conscious rat. *Naunyn-Schmiedebergs Arch. Pharmacol.* **341**, 381-384.

Chaouloff, F., Laude, D. and Baudrie, V. (1990b). Effects of the 5-HT1C/5-HT2 receptor agonists DOI and alpha-methyl-5-HT on plasma glucose and insulin levels in the rat. *Eur. J. Pharmacol.* **187**, 435-443.

Chaouloff, F., Laude, D. and Baudrie, V. (1990c). Ganglionic transmission is a prerequisite for the adrenaline-releasing and hyperglycemic effects of 8-OH-DPAT. *Eur. J. Pharmacol.* **185**, 11-18.

Chaouloff, F., Gunn, S. H. and Young, J. B. (1992). Central 5-hydroxytryptamine2 receptors are involved in the adrenal catecholamine-releasing and hyperglycemic effects of the 5-hydroxytryptamine indirect agonist d-fenfluramine in the conscious rat. *J. Pharmacol. Exp. Ther.* **260**, 1008-1016.

Davson, H. and Segal, M. B. (1996). *Physiology of the CSF and Blood-Brain Barriers*. CRC Press, Inc.

De Pedro, N., Pinillosm, M. L., Valenciano, A. I., Alonso-Bedate, M. and Delgado, M. J. (1998). Inhibitory effect of serotonin on feeding behavior in goldfish: Involvement of CRF. *Peptides* **19**, 505-511.

Egashira, N., Okuno, R., Matsushita, M., Abe, M., Mishima, K., Iwasaki, K., Nishimura, R., Oishi, R. and Fujiwara, M. (2008). Aripiprazole inhibits marble-burying behavior via 5-hydroxytryptamine (5-HT)1A receptor-independent mechanisms. *Eur. J. Pharmacol.* **592**, 103-108.

Figueroa, R. I., Rodríguez-Sabaris, R., Aldegunde, M. and Soengas, J. L. (2000). Effects of food deprivation on 24-h changes in brain and liver carbohydrate and ketone body metabolism of rainbow trout. *J. Fish Biol.* **57**, 631-646.

Freitas, R. M., Sousa, F. C., Viana, G. S. and Fonteles, M. M. (2006). Effect of gabaergic, glutamatergic, antipsychotic and antidepressant drugs on pilocarpine-induced seizures and status epilepticus. *Neurosci. Lett.* **408**, 79-83.

Fritsche, R., Reid, S. G., Thomas, S. and Perry, S. F. (1993). Serotonin-mediated release of catecholamines in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **178**, 191-204.

Gallo, V. P. and Civinini, A. (2003). Survey of the adrenal homolog in teleosts. *Int. Rev. Citol.* **230**, 89-187.

Guscott, M. R., Egan, E., Cook, G. P., Stanton, J. A., Beer, M. S., Rosahl, T. W., Hartmann, S., Kulagowski, J., McAllister, G., Fone, K. C. et al. (2003). The hypothermic effect of 5-CT in mice is mediated through the 5-HT7 receptor. *Neuropharmacol.* **44**, 1031-1037.

Hagan, J. J., Slade, P. D., Gaster, L., Jeffrey, P., Hatcher, J. P. and Middlemiss, D. N. (1997). Stimulation of 5-HT1B receptors causes hypothermia in the guinea pig. *Eur. J. Pharmacol.* **331**, 169-174.

Hannon, J. and Hoyer, D. (2008). Molecular biology of 5-HT receptors. *Behav. Brain Res.* **195**, 198-213.

Iwama, G. K., Afonso, L. O. B. and Vijayan, M. M. (2004). Stress in fish. In *Aquanet Workshop on Fish Welfare, Canada,* pp. 1-9. Canada: B.C. Campbell River.

Janvier, J. J., Peyraud-Waitzenegger, M. and Soulier, P. (1996). Effects of serotonin on the cardio-circulatory system of the European eel (*Anguilla anguilla*) in vivo. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **166**, 131-137.

Jonnakuty, C. and Gragnoli, C. (2008). What do we know about serotonin?. *J. Cell. Physiol.* **217**, 301-306.

Lundquist, F. (1942). The blood-brain barrier in some freshwater teleosts. *Acta Physiol. Scand.* **4**, 201-206.

Montpetit, C. J. and Perry, S. F. (1999). Neuronal control of catecholamine secretion from chromaffin cells in the rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **202**, 2059-2069.

Morita, K., Hamamoto, M., Arai, S., Kitayama, S., Irifune, M., Kawahara, M., Kihira, K. and Dohi, T. (2005). Inhibition of serotonin transporters by cocaine and meprylcaine through 5-TH2C receptor stimulation facilitates their seizure activities. *Brain Res.* **1057**, 153-160.

Muller, E. E., Cocchi, D. and Forni, A. (1971). A central site for the hyperglycemic action of 2-deoxy-D-glucose in mouse and rat. *Life Sci.* **10**, 1057-1067.

Naylor, J. L., Widdowson, P. S., Simpson, M. G., Farnworth, M., Ellis, M. K. and Lock, E. A. (1995). Further evidence that the blood/brain barrier impedes paraquat entry into the brain. *Hum. Exp. Toxicol.* **14**, 587-594.

Niesink, R. J. and Van Ree, J. M. (1982). Antidepressant drugs normalize the increased social behaviour of pairs of male rats induced by short term isolation. *Neuropharmacol.* **21**, 1343-1348.

Perasso, L., Cupello, A., Lunardi, G. L., Principato, C., Gandolfoa, C. and Balestrinoa, M. (2003). Kinetics of creatine in blood and brain after intraperitoneal injection in the rat. *Brain Res.* **974**, 37-42.

Preston, J. E., Hameed, A.-S. and Malcolm, B. S. (1995). Permeability of the developing blood-brain barrier to 14C-mannitol using the rat in situ brain perfusion technique. *Dev. Brain Res.* **87**, 69-76.

Reid, S. G., Furimsky, M. and Perry, S. F. (1994). The effects of repeated physical stress or fasting on catecholamine storage and release in the rainbow trout, *Oncorhynchus mykiss*. *J. Fish Physiol.* **45**, 365-378.

Reid, S. G., Bernier, N. J. and Perry, S. F. (1998). The adrenergic stress response in fish: Control of catecholamine storage and release. *Comp. Biochem. Physiol.* **120C**, 1-27.

Ruibal, C., Soengas, J. L. and Aldegunde, M. (2002). Brain serotonin and the control of food intake in rainbow trout (*Oncorhynchus mykiss*): effects of changes in plasma glucose levels. *J. Comp. Physiol. A* **188**, 479-484.

Schechter, M. D. (1988). Use of TFMPP stimulus properties as a model of 5-HT1B receptor activation. *Pharmacol. Biochem. Behav.* **31**, 53-57.

Scotti de Carolis, A., Passarelli, F. and Pezzola, A. (1986). Study on the anticonvulsant activity of clonidine against pentylenetetrazol-induced seizures in rats: pharmacological evidence of alpha 2-adrenoceptors mediation. *Arch. Int. Pharmacodyn. Ther.* **282**, 209-218.

Soengas, J. L. and Aldegunde, M. (2002). Energy metabolism of fish brain. *Comp. Biochem. Physiol.* **131B**, 271-296.

Soengas, J. L. and Aldegunde, M. (2004). Brain glucose and insulin: effects on food intake and brain amines of rainbow trout. *J. Comp. Physiol. A* **190**, 641-649.

Sugimoto, Y., Yamada, J., Yoshikawa, T. and Horisaka, K. (1996a). Effects of the 5-HT2C/2B receptor agonist 1-(3-chlorophenyl) piperazine on plasma glucose levels of rats. *Eur. J. Pharmacol.* **307**, 75-80.

Sugimoto, Y., Yamada, J., Yoshikawa, T. and Horisaka, K. (1996b). The effects of peripheral serotonin2 (5-HT2) and serotonin3 (5-HT3) receptor agonists on blood glucose levels in rats. *Biol. Pharm. Bull.* **19**, 1384-1386.

Sugimoto, Y., Kimura, I., Watanabe, Y. and Yamada, J. (2001). The 5-HT1A receptor agonist 8-hydroxy-2-di-n-(propylamino)tetralin (8-OH-DPAT) induces hyperglucagonemia in rats. *Biol. Pharm. Bull.* **24**, 1191-1194.

Sundin, L., Davison, W., Forster, M. and Axelsson, M. (1998). A role of 5-HT2 receptors in the gill vasculature of the antarctic fish *Pagothenia borchgrevinki*. *J. Exp. Biol.* **201**, 2129-2138.

Uvnäs-Moberg, K., Ahlenius, S., Alster, P. and Hillegaart, V. (1996). Effects of selective serotonin and dopamine agonists on plasma levels of glucose, insulin and glucagon in the rat. *Neuroendocrinol.* **63**, 269-274.

Vitte, P. A., Harthe, C., Lestage, P., Claustrat, B. and Bobillier, P. (1988). Plasma, cerebrospinal fluid, and brain distribution of 14C-melatonin in rat: a biochemical and autoradiographic study. *J. Pineal Res.* **5**, 437-453.

Weidenfeld, J., Corcos, A. P., Wohlman, A. and Feldman, S. (1994). Characterization of the 2-deoxyglucose effect on the adrenocortical axis. *Endocrinol.* **134**, 1924-1931.

Wikstrom, M., Hill, R., Hellgren, J. and Grillner, S. (1995). The action of 5-HT on calcium-dependent potassium channels and on the spinal locomotor network in lamprey is mediated by 5-HT1A-like receptors. *Brain Res.* **678**, 191-199.

Winberg, S. and Nilsson, G. E. (1993). Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. *Comp. Biochem. Physiol.* **106C**, 597-614.

Winberg, S. and Nilsson, G. E. (1996). Multiple high-affinity binding sites for [3H]serotonin in the brain of a teleost fish, the arctic charr (*Salvelinus alpinus*). *J. Exp. Biol.* **199**, 2429-2435.

Yamada, J., Sugimoto, Y., Yoshikawa, T., Kimura, I. and Horisaka, K. (1995). The involvement of the peripheral 5-HT2A receptor in peripherally administered serotonin-induced hyperglycemia in rats. *Life Sci.* **57**, 819-825.

Yamada, J., Sugimoto, Y., Yoshikawa, T. and Horisaka, K. (1997). Hyperglycemia induced by the 5-HT receptor agonist, 5-methoxytryptamine, in rats: involvement of the peripheral 5-HT2A receptor. *Eur. J. Pharmacol.* **323**, 235-240.

Yamada, J., Sugimoto, Y., Noma, T. and Yoshikawa, T. (1998). Effects of the non-selective 5-HT receptor agonist, 5-carboxamidotryptamine, on plasma glucose levels in rats. *Eur. J. Pharmacol.* **359**, 81-86.