Am J Physiol Regul Integr Comp Physiol 315: R1154–R1166, 2018.
First published October 10, 2018; doi:10.1152/ajpregu.00137.2018.

RESEARCH ARTICLE | *Cardiovascular and Renal Integration*

The serotonin transporter and nonselective transporters are involved in peripheral serotonin uptake in the Gulf toadfish *Opsanus beta*

Molly H. B. Amador and M. Danielle McDonald

Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida

Submitted 7 May 2018; accepted in fina form 9 October 2018

Amador MH, McDonald MD. The serotonin transporter and nonselective transporters are involved in peripheral serotonin uptake in the Gulf toadfish *Opsanus beta*. *Am J Physiol Regul Integr Comp Physiol* 315: R1154–R1166, 2018. First published October 10, 2018; doi:10.1152/ajpregu.00137.2018.—In mammals, circulating serotonin [5-hydroxytryptamine (5-HT)] is sequestered by platelets via the 5-HT transporter (SERT) to prevent unintended signaling by this potent signaling molecule. Teleost fis appear to lack a similar circulating storage pool, although the diverse effects of 5-HT in teleosts likely necessitate an alternative method of tight regulation, such as uptake by peripheral tissues. Here, a 5-HT radiotracer was used to explore the 5-HT uptake capacity of peripheral tissues in the Gulf toadfish *Opsanus beta*, and to elucidate the primary excretion routes of 5-HT and its metabolites. Pharmacological inhibition of SERT and other transporters enabled assessment of the SERT dependence of peripheral 5-HT uptake and excretion. The results indicated a rapid and substantial uptake of 5-HT by the heart atrium, heart ventricle, and gill that was at least partly SERT dependent. The results also supported the presence of a partial blood-brain barrier that prevented rapid changes in brain 5-HT content despite fluctuatin plasma 5-HT concentrations. The renal pathway appeared to be the dominant excretory route for 5-HT and its metabolites over shorter time frames (up to ~30 min), but hepatic excretion was substantial over several hours. SERT inhibition ultimately reduced the excretion of 5-HT and its metabolites by urinary, biliary, and/or intestinal pathways. In addition, branchial excretion of 5-HT and its metabolites could not be ruled out. In summary, this study reveals that the toadfis heart and gill play active roles in regulating circulating 5-HT and yields important insights into the control of peripheral 5-HT in this teleost fish

bupropion; decynium-22; fluoxetine 5-hydroxytryptamine; serotonin transport

INTRODUCTION

Because of the ubiquity of serotonin [5-hydroxytryptamine (5-HT)] signaling in the animal kingdom, 5-HT receptors and the 5-HT transporter (SERT) are highly conserved among vertebrates (2, 44, 58, 60, 79, 93, 101). As in mammals, 5-HT in teleost fis is vasoactive; its most well-studied effect is the constriction of the branchial vasculature (22, 23, 38, 70, 77). Among other functions (48), 5-HT can also alter heart rate (58), increase ventilation (38, 50), stimulate cortisol secretion (43, 58, 59, 98), influenc aggression, movement, and anxiety (48, 63, 64, 96, 97), affect reproduction (71), feeding (15a), and gut motility (89), potentially mediate oxygen sensing and the hypoxia response (6, 10, 39, 85), and, in Gulf toadfis in particular, activate a unique urea pulsing mechanism (44, 54, 100).

Given such widespread influence it might be expected that circulating 5-HT needs to be as tightly controlled in fis as it is in mammals; however, fis lack the platelets used by mammals as a critical 5-HT storage pool (14, 19, 61). Instead, fis possess nucleated thrombocytes, which are considered ancestral to platelets and perform similar functions in blood clot formation (11, 47, 87) but do not appear to store 5-HT (20), although 5-HT immunoreactivity has recently been reported in zebrafis thrombocytes from cardiac vessels (84). It is also possible that other blood cell types could play a role in 5-HT storage, as Ferriere et al. (21) demonstrated a 5-HT uptake mechanism in rainbow trout lymphocytes. However, Caamaño-Tubío et al. (11) showed that 5-HT concentrations in rainbow trout plasma do not differ significantl from those in whole blood, and toadfis whole blood contains negligible amounts of the transcript of SERT (2), which is responsible for extracellular 5-HT uptake.

Despite their apparent lack of a circulating storage pool, teleosts have plasma 5-HT concentrations (2, 11, 31, 51, 62) similar to those of mammals (approximately in the 10^{-8}–10^{-9} M range) (32, 46). This may not be simply due to lower levels of 5-HT synthesis or release in fish for example, the isolated teleost intestine has been found to spontaneously release 5-HT at a rate similar to that of isolated mammalian intestines (4, 26, 34). Rather, the maintenance of low plasma 5-HT concentrations in fis suggests that other processes, such as tissue 5-HT uptake, metabolism, or excretion, must be regulating circulating 5-HT levels (11).

There are several lines of evidence indicating the potential for teleost tissues to remove 5-HT from the bloodstream. In the toadfis and goldfish SERT mRNA expression has been detected in every tissue examined (2, 60), suggesting the possibility for 5-HT uptake in tissues throughout the periphery. In the zebrafis atrium, at least two cell types appear to sequester 5-HT (84), which is consistent with the remarkably high relative mRNA expression of SERT in the toadfis heart (2). In addition, all teleosts investigated have neuroepithelial cells in the gill that store 5-HT in vesicles (6, 16, 40, 102). Furthermore, an isolated gill preparation can remove at least 40% of 5-HT from perfusate (66), and significan SERT mRNA expression has been measured in the gill (2, 3, 12, 60), highlighting a potential role for the gill in 5-HT management. Metabolism of 5-HT into its main metabolite, 5-hydroxyindoleacetic

Address for reprint requests and other correspondence: M. H. B. Amador, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Univ. of Miami, Miami, FL 33149 (e-mail: mbroome@rsmas.miami.edu).

acid (5-HIAA), by the gill, liver, kidney, and intestine (11, 17, 66, 82) may also play a role in keeping plasma 5-HT levels low.

Given the uncertainties regarding the control of circulating 5-HT in teleosts, the objective of the current study was to determine the capacity for SERT-expressing tissues in the Gulf toadfish (*Opsanus beta*) to take up 5-HT from the circulation. This teleost has been well studied because of its extremely hardy nature (30, 88, 94), its aglomerular kidney (52, 53, 55, 56), its ability to switch from ammoniotely to ureotely (36, 91), and its branchial urea pulsing mechanism (91, 99). We hypothesized that tissues with high SERT mRNA expression in toadfish or with previously documented roles in 5-HT extraction in teleosts, specifically the heart and gill, would demonstrate SERT-dependent uptake of plasma 5-HT, as evidenced by sensitivity to the SERT inhibitor fluoxetin (FLX). Potential excretory pathways were also examined, and it was hypothesized that *1*) the kidney and liver would play the major roles in excretion of 5-HT and/or its metabolite 5-HIAA and that *2*) excretion of 5-HT or its metabolites would be transporter dependent.

MATERIALS AND METHODS

Experimental Animals

Gulf toadfish (*O. beta*) were caught as roller trawl bycatch by local shrimpers in Biscayne Bay, Florida (Florida Fish and Wildlife Conservation Commission Special Activity License no. SAL-16–0729-SR). Upon their arrival to the laboratory, fish were exposed to freshwater for 15 min, returned to seawater, and subsequently treated on three consecutive days with a final concentration of 0.1 mg/l malachite green in 30 mg/l formalin to treat and prevent infection by ectoparasites. Fish were housed in aerated, flow-through 20-gallon aquaria supplied with filtered seawater from Biscayne Bay and were fed raw (previously frozen) shrimp weekly to satiation. Water temperatures ranged from 18°C to 22°C. All protocols were carried out with the approval of the University of Miami Institutional Animal Care and Use Committee.

Experimental Procedures

Series 1: 5-HT uptake inhibition by FLX only. Toadfish (n = 10) were anesthetized in buffered (pH 8.2) 1 g/l tricaine methanesulfonate (MS-222; Western Chemical, Ferndale, WA), and each fish was surgically fitted with an indwelling caudal vessel (arterial or venous) catheter (Intramedic PE 50 tubing; Becton Dickinson, Franklin Lakes, NJ) filled with heparinized saline (150 mM NaCl with 50 IU/ml sodium heparin; Sigma-Aldrich) and an intraperitoneal catheter (Intramedic PE 160 tubing; Becton Dickinson) filled with peanut oil, as described previously (54). Fish were allowed to recover in individual plastic chambers with aerated, flow-through seawater for ~36 h before experiments.

Several pilot experiments were completed so that we could ascertain the appropriate incubation times ($t_{\mathrm{incubation}}$) for FLX (pilot 1: 22 h; pilot 2: 75 min; pilot 3: 30 min) and equilibration times ($t_{\mathrm{equilibration}}$) for [^{3}H]5-HT (pilot 1: 2 h; pilot 2: 1 h; pilot 3: 15 min). Based on the information gained by these pilot experiments, we decided on our experimental method. After fish recovered from surgery, they were implanted with coconut oil only or with 50 μg/g FLX (as FLX hydrochloride; Toronto Research Chemicals; North York, Canada) in coconut oil (n = 5 per treatment; average fish mass 80.7 ± 4.3 g) via intraperitoneal catheter. After a $t_{\mathrm{incubation}}$ = 5 min, fish were injected with 0.1 μCi/g [^{3}H]5-HT creatinine sulfate (27.7 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) via caudal vessel catheter with a Hamilton syringe (t = 0). 5-HT creatinine sulfate is a naturally occurring complex, the synthetic form of which produces characteristic 5-HT responses in vivo (73, 83). Blood was drawn into the catheter several times after injection to ensure full delivery of the isotope. At t = 2 min and every 5 min thereafter for 30 min ($t_{\mathrm{equilibration}}$ = 32 min), 150- to 200-μl blood samples were collected. An aliquot (20 μl) of plasma was analyzed for [^{3}H]5-HT radioactivity. The remainder was flash frozen in liquid nitrogen and stored at −80°C for later analysis of 5-HT concentrations. Fish were euthanized at t = 32 min in 3 g/l MS-222.

After euthanization, tissues (cerebellum, rest of brain, gill arches, sinus venosus, atrium, ventricle, bulbus arteriosus, gall bladder, spleen, stomach, intestine, swim bladder, liver, gonad, bladder, kidney, skin, and muscle) were harvested. The cerebellum was collected and analyzed separately from the rest of the brain because of its markedly higher SERT mRNA expression (~300-fold higher) than other brain regions (2). The heart was removed first to stop circulation of isotope, rinsed with 150 mM saline, and gently pressed to remove blood from the lumen. After collection, tissues were digested in 5 volumes (5 μl/mg) of 1 M nitric acid for 48 h at 70°C; samples were vortexed after 24 and 48 h. Tissue digests were then centrifuged 5 min at 1,500 g to enable the collection of supernatant for measurement of radioactivity.

Series 2: 5-HT uptake inhibition by multiple drugs. To block the uptake of 5-HT through transporters other than SERT (15, 28) and thus enable a more accurate examination of SERT-mediated uptake, additional uptake experiments were performed. Bupropion (BUP) (as BUP hydrochloride), a dopamine transporter (DAT) and norepinephrine transporter (NET) inhibitor, and decynium-22 (D-22) (as 1,1′-diethyl-2,2′-cyanine iodide, 97%), an inhibitor of organic cation transporters and the plasma monoamine transporter, were purchased from VWR International (Radnor, PA). D-22 stock solution (0.67 μg/μl) and solutions of BUP (50 μg/μl) and FLX (100 μg/μl) were created in 100% ethanol. The D-22 stock solution was further diluted 1:2.33 in ethanol to create the final drug solution (0.2 μg/μl). Drug solutions were stored in aliquots at −20°C for use on subsequent days. One day before each experiment, coconut oil vehicle in microcentrifuge tubes was overlaid with 100% ethanol (control), BUP (10 μg/g fish and D-22 working stock (0.01 μg/g fish) or BUP (10 μg/g fish) D-22 working stock (0.01 μg/g fish) and FLX (50 μg/g fish) Although effective doses of BUP and D-22 have not been established for fish the final dose of BUP has been found to elicit antidepressant effects in rats (13), and that of D-22 has been reported to be the lowest effective therapeutic dose in rats (5, 45). Ethanol was added to control tubes and to tubes overlaid with BUP and D-22 to ensure an equal overlay volume in all tubes. Tubes were left open overnight in a water bath at ~30°C to facilitate the evaporation of ethanol while maintaining the vehicle in a liquid state. After evaporation, tubes were vortexed and their contents aspirated with a syringe fitted with an 18-gauge needle to create a drug slurry.

Fish (n = 18; average fish mass 66.3 ± 2.1 g) were surgically fitted with caudal vessel (arterial or venous; 1 per fish and intraperitoneal catheters as described above. After 36 h of recovery, fish were implanted via intraperitoneal catheter with 5 μl/g coconut oil (n = 6), BUP and D-22 in coconut oil (+B +D; n = 6), or BUP, D-22, and FLX in coconut oil (+B +D +FLX; n = 6). After a $t_{\mathrm{incubation}}$ = 5 min, fish were injected via caudal vessel catheter with 0.1 μCi/g [^{3}H]5-HT (36.5 Ci/mmol) in 150 mM saline (1 μl/g injection volume). After a $t_{\mathrm{equilibration}}$ = 2 min, 150–200-μl blood samples were collected and processed as described above. Fish were immediately anesthetized in 4 g/l MS-222, and tissues were collected and digested as described above (the sinus venosus was included with the atrium).

Series 3: determination of 5-HT excretory pathways at discrete time points. To assess the relative contributions of three potential 5-HT excretory pathways [bile, urine, and external water (the latter being a combination of intestinal, urinary, and gill output] in individual fish at a given point in time, fish (n = 29; average fish mass 45.7 ± 1.7 g) were first wrapped in paper towels and injected with

0.02–0.1 μCi/g of [^{3}H]5-HT (27.7 Ci/mmol) in 150 mM saline (injection volume 1 μl/g) via caudal vessel puncture using a syringe fitte with a 23-gauge needle and rinsed with heparinized saline. Fish were then placed in aerated plastic chambers with 1–1.5 liters of seawater and left undisturbed for up to 16 h. At 0.25, 0.5, 1, 2, 4, 8, and 16 h, a water sample was taken and fis (n = 4 per time point) were removed from their chambers; a 100- to 200-μl blood sample was taken and placed on ice. Fish were quickly euthanized in 4 g/l MS-222, and bile and urine were collected. Blood samples were processed as described above. An aliquot (20 μl) of bile, urine, and water was analyzed for radioactivity. Water samples were stored at −20°C until analysis of urea and ammonia concentrations.

Series 4: divided chamber experiment. For a more accurate assessment of the relative contributions of the gills versus the kidney and liver to 5-HT excretion in individual fis over time, toadfis (n = 15; average fis mass 72.2 ± 3.1 g) fed one day previously were anesthetized in buffered 1 g/l MS-222 and fitte with indwelling caudal vessel catheters (arterial or venous; 1 catheter per fish fille with heparinized saline as described above. After 36 h of recovery in individual aerated chambers with flow-throug seawater, fis were again anesthetized in buffered 1 g/l MS-222 and injected intraperitoneally with coconut oil only (control; n = 5), BUP and D-22 in coconut oil (+B +D; n = 5), or BUP, D-22, and FLX in coconut oil (+B +D +FLX; n = 5) using a gastight Hamilton syringe fitte with an 18-gauge needle. Fish were wrapped in paper towels and placed on ice briefl (~30 s) to solidify implants.

After implantation, fis were fitte with rubber dams and positioned in divided chambers as described by Wood et al. (99). Briefly each fis was pushed through a small hole (~1 inch in diameter) cut in a square Elasti-Dam rubber dam (Hygenic, Akron, OH) until the dam rested behind the opercula and pectoral fins Sutures were made with 2–0 suture silk and a tapered (noncutting) surgical needle to secure the dam to the skin of the fish Each fis was then positioned spanning two acrylic half-chambers; the front and rear of the fis were supported by mesh platforms in the chambers, and the rubber dam was stretched between the two half-chambers to form a septum. The half-chambers were secured together using metal clips and a neoprene gasket as a seal. The whole divided chamber apparatus was then placed in a larger, darkened plastic outer container, and the anterior and posterior half-chambers were fille with known volumes of seawater (anterior chambers: 0.95–1.0 liter, posterior chambers: 1.0–1.25 liters). Air stones were placed in the anterior and posterior chambers to ensure thorough mixing of water in each half-chamber. Catheters were secured outside of the outer containers to enable blood sampling without disturbance to the fish and holes in the lids of the outer container likewise enabled unobtrusive water sampling from each half-chamber.

After a recovery period of ~40–50 min, fis were injected (t = 0) with 0.1 μCi/g [^{3}H]5-HT (36.5 Ci/mmol) in 150 mM NaCl (injection volume 1 μl/g) via the caudal vessel catheter. Blood samples were taken at t = 0.25, 0.5, 1, 2, 4, 8, and 16 h. Water samples (1 ml) were also taken from anterior and posterior half-chambers at each time point. Blood and water samples were immediately placed on ice until further processing. After the last water samples were taken, food coloring was added to the posterior chamber to ensure the absence of any leaks between chambers. No leaks were found in any of our trials. In a separate pilot experiment, we completed a more robust test for leaks using radioisotope. Toadfis (n = 3; average fis mass 72.4 ± 8.4 g) were placed into the divided chamber setup and allowed to acclimate for ~30–40 min (36.7 ± 5.8 min) before 1 μCi of [^{3}H]5-HT was added to the posterior half-chamber. After 16 h, water samples (4 ml) were collected from the anterior and posterior half-chambers. Isotope was undetectable in the anterior half-chambers of two of the three setups 16 h after the posterior half-chamber was spiked. In the third setup, the amount of isotope in the anterior half-chamber was 0.14% of that in the posterior chamber.

Analytical Techniques and Calculations

Samples of plasma, tissue digest supernatant, water, bile, and urine (20 μl) from all experiments were added to plastic scintillation vials containing 4 ml Ultima Gold liquid scintillation cocktail (PerkinElmer, Waltham, MA), and vials were shaken vigorously. For *series 1* and *3*, vials sat in the dark overnight before radioactivity was counted for 1 min [as counts per minute (cpm)] in a Wallac 1415 liquid scintillation counter (Wallac Oy, Turku, Finland) using MultiCalc software (v. 1.52, Wallac Oy) with no quench correction (because of limitations of this equipment). For *series 2* and *4* experiments that were done at a later time, vials were counted for 1 min [as cpm (*series 4*) or disintegrations per minute (dpm) (*series 2*)] in a PerkinElmer Tri-Carb 2910TR liquid scintillation counter (PerkinElmer) using QuantaSmart software (PerkinElmer). Plasma 5-HT concentrations were measured using an ELISA kit (ALPCO Diagnostics, Salem, NH), as used previously for toadfis plasma (62). Urea was quantifie using the diacetyl monoxime method (72), and ammonia was quantifie using the indophenol blue method (37).

For all experiments, average cumulative plasma-specifi activities were calculated for each sampling time (or for each fish when only one blood sample was taken) based on the plasma radioactivity and the measured plasma 5-HT concentrations as in the following equation:

$$SA_P = \frac{a}{b} \quad (1)$$

where SA_P is the plasma specifi activity, a is the plasma radioactivity (in cpm/ml or dpm/ml), and b is the total 5-HT concentration in the plasma (in ng/ml).

In *series 1*, a one-phase exponential decay curve was fitte to the plasma data for repeatedly sampled control fis in Prism software (v. 7; GraphPad, La Jolla, CA). Radioactivity of all digested tissues (FLX-only and multiple-drug uptake experiments) was converted into total 5-HT uptake per gram of tissue based on the average specifi activity for each fish' plasma, according to the following equation:

$$U = \frac{c}{SA_P} \quad (2)$$

where U is the tissue 5-HT uptake and c is the radioactivity (in cpm or dpm) per gram of tissue (wet weight) (27). Relative uptake, or uptake corrected for plasma 5-HT concentrations, was calculated according to the following equation:

$$RU = \frac{U}{b_{avg}} \quad (3)$$

where RU is the relative tissue uptake and b_{avg} is the average plasma 5-HT concentration over all time points.

Statistics

All data were assessed for normality using the Shapiro-Wilk test and, if possible, nonnormal data were log-transformed to meet normality assumptions. For *series 1* experiments, differences in plasma 5-HT concentrations over time were analyzed by repeated measures Friedman test with Dunn's multiple comparisons test or one-way repeated measures ANOVA with Dunnett's multiple comparisons test. Unpaired Student's t-tests or Mann-Whitney U tests were used to compare mean plasma 5-HT concentrations between control and FLX-treated fis at each time point and to compare 5-HT uptake between control and FLX-treated fis within each tissue. 5-HT uptake across control tissues for *series 1*, across control tissues for *series 2*, and across treatments within individual tissues or fluid for *series 2* was analyzed by one-way ANOVA with Tukey's multiple comparisons tests. For the *series 3* experiments, [^{3}H] accumulation in bile and urine was analyzed by two-way ANOVA (with flui type and treatment as factors) with Sidak's and Tukey's multiple comparisons tests. Differences in [^{3}H] accumulation in water between time points were assessed by one-way ANOVA with Tukey's multiple comparisons test. For *series 4*, differences in plasma 5-HT across multiple drug treatments and time points were assessed by repeated measures two-way ANOVA with Tukey's and Dunnett's multiple comparisons tests, respectively. Repeated measures one-way ANOVAs with Dunnett's multiple comparisons tests or, for nonnormal data, Friedman tests with Dunn's multiple comparisons tests were used to analyze differences in [^{3}H] accumulation in each compartment type over time for each treatment. Differences in overall [^{3}H] excretion rates were assessed by two-way ANOVA with Sidak's multiple comparisons tests. Statistical significanc was define as $P < 0.05$. Data are presented as means ± SE.

RESULTS

Series 1: 5-HT uptake inhibition by FLX only. Plasma 5-HT concentrations in fis treated with 50 μg/g FLX were 1.5-fold greater than those of control fis by 7 min postisotope injection, reaching the maximal difference of 3.4-fold greater by 22 min postinjection (Fig. 1*A*). When uptake was considered on a whole tissue basis, the greatest uptake occurred in the intestine > kidney > liver > swim bladder > stomach > gill > heart ventricle (Table 1). A comparison of 5-HT uptake across control fis tissues on a per-gram-tissue basis after the 32-min isotope equilibration period revealed considerably greater uptake in most peripheral tissues than in the brain (minus the cerebellum); for example, uptake in the heart atrium and ventricle was eight- and sixfold greater than in the brain, whereas uptake in the gill arches was four- to fivefol greater than in the brain (Fig. 1*B*). The greatest uptake per gram of tissue, 28-fold higher than in the brain, occurred in the kidney (Fig. 1*B*).

Surprisingly, uptake of 5-HT per gram of tissue was significantly greater in the atrium, gall bladder, intestine, gonad, bladder, kidney, and skin of FLX-treated fis than in control fish with no significan effect of FLX treatment in other tissues (Fig. 1*B*). Previous pilot trials using different $t_{incubation}$ and $t_{equilibration}$ had yielded similar results with respect to the effects of FLX (data not shown). However, the relative 5-HT uptake, which removed the influenc of systemically elevated 5-HT in

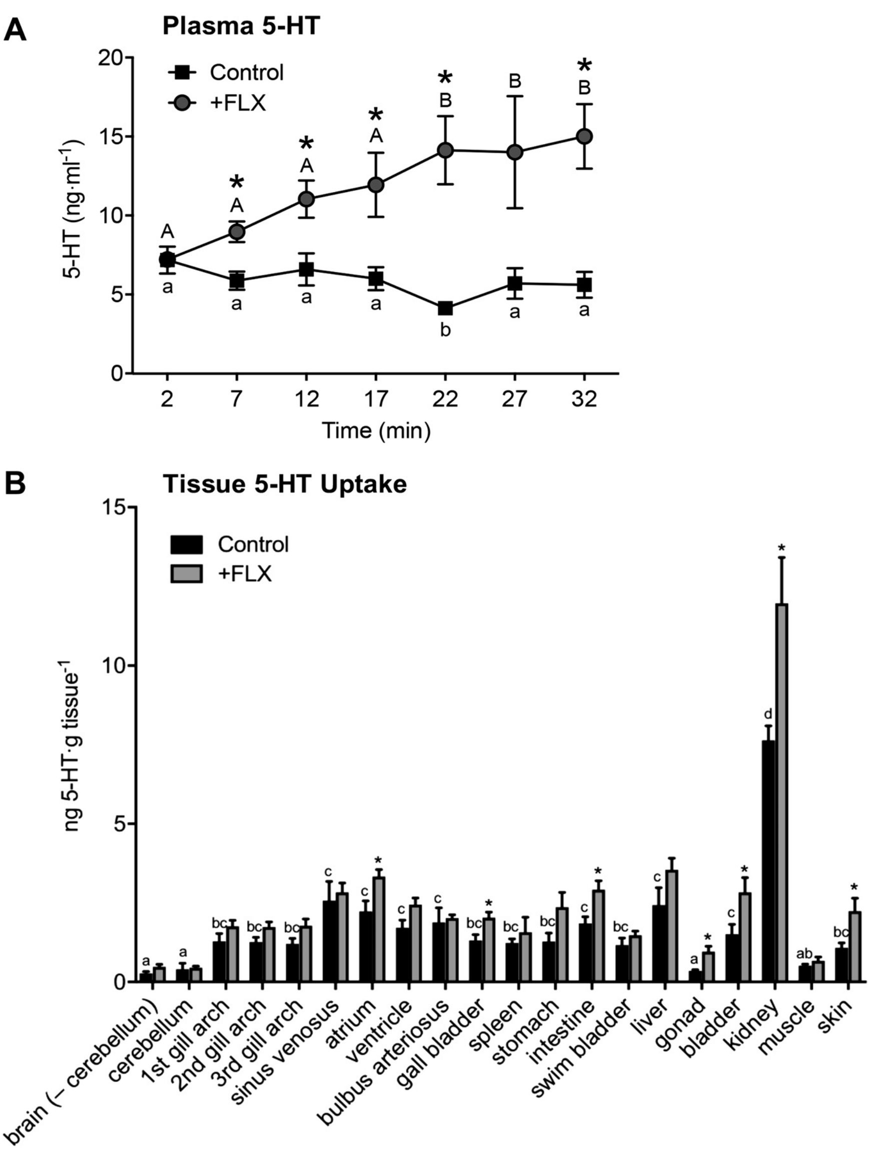

Fig. 1. Plasma 5-hydroxytryptamine (5-HT) concentrations (*A*) and tissue uptake of 5-HT (*B*) after treatment with coconut oil (control) or 50 μg/g fluoxetin (FLX). Values are means ± SE. Different letters denote a significan difference (within treatment) compared with concentrations in *A* at $t = 2$ min [$P < 0.05$; repeated measures Friedman's test with Dunn's multiple comparisons test (control) or repeated measures one-way ANOVA with Dunnett's multiple comparisons test (+FLX)] or other control tissues (*B*) ($P < 0.05$; one-way ANOVA with Tukey's multiple comparisons test; $n = 5$, except for bladder where $n = 4$). *Significan difference from controls at the same time point (*A*) [$P < 0.05$; Student's *t*-test or Mann-Whitney *U* test ($t = 12$ min); $n = 5$] or from controls within the same tissue (*B*) [$P < 0.05$; Student's *t*-test or Mann-Whitney *U* test (muscle only); $n = 5$, except for bladder and +FLX sinus venosus where $n = 4$].

Table 1. *Whole tissue 5-HT uptake for control fish 32 min after isotope injection in order of increasing uptake*

Tissue	5-HT Uptake, ng
Cerebellum	0.001 ± 0.000 (5)^{a}
Sinus venosus	0.007 ± 0.002 (5)^{b}
Brain (−cerebellum)	0.021 ± 0.004 (5)^{c}
Bulbus arteriosus	0.033 ± 0.010 (5)^{c,d}
Bladder	0.037 ± 0.008 (4)^{c,d,e}
Gall bladder	0.040 ± 0.008 (5)^{c,d,e}
Atrium	0.042 ± 0.007 (5)^{c,d,e}
Spleen	0.061 ± 0.010 (5)^{c,d,e}
Gonad	0.084 ± 0.022 (5)^{d,e}
Ventricle	0.090 ± 0.017 (5)^{e,f}
Third gill arch	0.237 ± 0.034 (5)^{f,g}
Second gill arch	0.284 ± 0.034 (5)^{g}
First gill arch	0.253 ± 0.049 (5)^{f,g}
Stomach	1.170 ± 0.303 (5)^{h}
Swim bladder	1.688 ± 0.360 (5)^{h}
Liver	2.833 ± 0.554 (5)^{h}
Kidney	2.837 ± 0.308 (5)^{h}
Intestine	3.180 ± 0.389 (5)^{h}

Values are means ± SE (n). 5-HT, 5-hydroxytryptamine. Entries not sharing a letter are significantl different ($P < 0.05$; one-way ANOVA with Tukey's multiple comparisons test).

FLX-treated fish revealed significantl lower 5-HT uptake in many tissues, including the gill and heart, of FLX-treated fis (Table 2).

Series 2: 5-HT uptake inhibition by multiple drugs. To better ascertain the influenc of circulating 5-HT concentrations and the role of other transporters in 5-HT uptake, fis were treated with the dopamine (DAT) and norepinephrine (NET) reuptake inhibitor BUP and the organic cation and plasma monoamine transport inhibitor D-22 (+B +D) or with these drugs in addition to FLX (+B +D +FLX). +B +D treatment did not significantl alter plasma 5-HT concentrations compared with those of control fis (Fig. 2*A*); however, as in fis treated with FLX alone (Fig. 1*A*), the addition of FLX to the other two drugs (+B +D +FLX) caused a 2.8-fold increase in plasma 5-HT compared with the levels of controls (Fig. 2*A*). On a whole tissue basis, the greatest uptake occurred in the kidney > heart ventricle > liver > intestine > swim bladder > gill > stomach (Table 3). A comparison of 5-HT uptake across tissues on a per gram tissue basis after this shorter 2-min isotope equilibration period in control fis revealed that the highest uptake occurred in the heart atrium and ventricle, which demonstrated ~155- and 136-fold greater uptake than in the brain, respectively (Fig. 2*B*), rather than in the kidney, as in the experiment with a longer isotope incubation period (*series 1*). However, uptake in the kidney remained elevated compared with the brain (44-fold greater; Fig. 2*B*). Uptake in the gill was 18-fold greater than that in the brain (Fig. 2*B*).

Brain 5-HT uptake per gram of tissue was not altered by +B +D treatment or by +B +D +FLX treatment (Fig. 3*A*). In the gill, however, treatment with all three drugs decreased 5-HT uptake by 62% compared with the gills of control fish whereas uptake in gills of fis treated with +B +D alone was not significantl different than that in either control or +B +D +FLX fis (Fig. 3*B*). Within the heart, the bulbus arteriosus showed no significan changes in 5-HT uptake with drug treatment ($P = 0.07$; Fig. 3*C*). +B +D treatment did not alter uptake in the atrium, but it decreased uptake in the ventricle by 54% compared with controls (Fig. 3*C*). However, in +B +D +FLX fish 5-HT uptake in both the atrium and the ventricle was 89% lower than in controls (Fig. 3*C*).

Tissues involved in the excretion of 5-HT or its main metabolite, 5-HIAA (the kidney, liver, urinary bladder, and gall bladder), demonstrated trends that contrasted with those of the gill, atrium, and ventricle. In all four excretory tissues, there was an overall effect of treatment, with an apparent increase in 5-HT uptake in +B +D +FLX fis compared with uptake in fis treated with +B +D alone, and in the case of the urinary bladder compared with uptake in controls (Fig. 3*D*). The remaining tissues demonstrated trends similar to those of excretory tissues (Fig. 3*E*). When treatments were pooled, overall [^{3}H] accumulation in urine was 2.4-fold greater than in the bile (Fig. 3*F*).

Series 3: determination of 5-HT excretory pathways at discrete time points. Spot sampling of urine, bile, and water over a 16-h isotope equilibration period showed that [^{3}H] in urine was detectable within 0.25 h of [^{3}H]5-HT injection and was significantl greater than initial values at 2, 4, 8, and 16 h, peaking at 4 h (Tale 4). Biliary accumulation of [^{3}H] was also significantl greater than initial values at 2, 4, 8, and 16 h (Table 4). There was a 1.4-fold greater accumulation of [^{3}H] in the urine than in the bile of fis at 0.5 h, but differences were not significan at other time points (Table 4). Accumulation of [^{3}H] in the external water appeared to peak by 4 h, but the increase was not significan (Table 4).

Series 4: divided chamber experiment. In the 16-h divided chamber experiment, plasma 5-HT was 1.5-fold higher in fis treated with +B +D +FLX than in fis treated with +B +D alone at 0.25 h and 1.9- and 1.6-fold higher than in both control and +B +D fish respectively, at 0.5 h (Fig. 4*A*). However, there were no significan differences in plasma 5-HT among treatments at any subsequent time points. By the end of the 16-h experiment, plasma 5-HT was 44% lower than initial (t =

Table 2. *Relative uptake by tissues in control and FLX-treated fish 32 min after isotope injection*

Tissue	Relative Uptake	
	Control	+FLX
Brain (−cerebellum)	0.04 ± 0.00 (5)	0.04 ± 0.00 (5)
Cerebellum	0.06 ± 0.02 (5)	0.04 ± 0.00 (5)
First gill arch	0.21 ± 0.03 (5)	0.15 ± 0.01 (5)
Second gill arch	0.22 ± 0.01 (5)	0.15 ± 0.01 (5)*
Third gill arch	0.20 ± 0.01 (5)	0.15 ± 0.01 (5)*
Sinus venosus	0.43 ± 0.10 (5)	0.25 ± 0.03 (4)
Atrium	0.38 ± 0.03 (5)	0.29 ± 0.02 (5)*
Ventricle	0.29 ± 0.02 (5)	0.21 ± 0.01 (5)*
Bulbus arteriosus	0.31 ± 0.05 (5)	0.18 ± 0.02 (5)*
Gall bladder	0.22 ± 0.01 (5)	0.18 ± 0.02 (5)*
Spleen	0.21 ± 0.01 (5)	0.13 ± 0.03 (5)*
Stomach	0.21 ± 0.03 (5)	0.19 ± 0.03 (5)
Intestine	0.32 ± 0.03 (5)	0.25 ± 0.01 (5)*
Swim bladder	0.20 ± 0.04 (5)	0.13 ± 0.02 (5)
Liver	0.40 ± 0.05 (5)	0.31 ± 0.04 (5)
Gonad	0.06 ± 0.01 (5)	0.08 ± 0.01 (5)
Bladder	0.28 ± 0.05 (4)	0.25 ± 0.04 (4)
Kidney	1.33 ± 0.08 (5)	1.05 ± 0.15 (5)
Muscle	0.09 ± 0.01 (5)	0.06 ± 0.01 (5)*
Skin	0.18 ± 0.02 (5)	0.18 ± 0.02 (5)

Values are means ± SE (n). FLX, fluoxetine *Significan difference from controls ($P < 0.05$; Student's t-test).

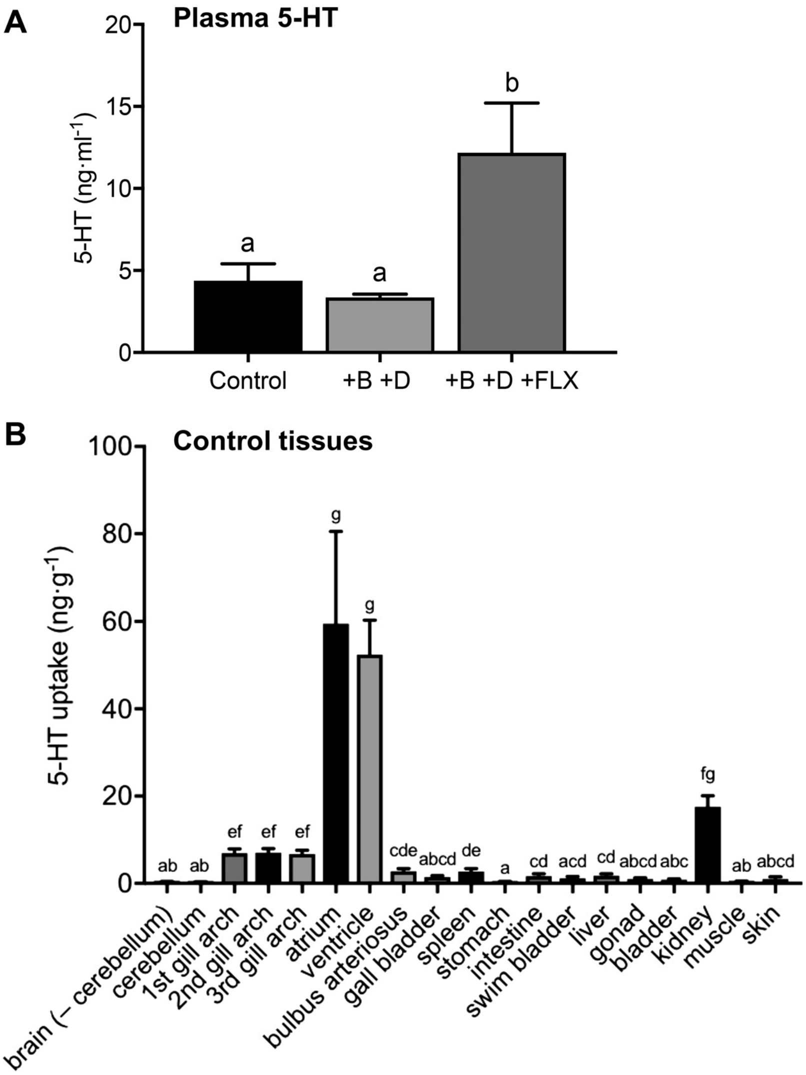

Fig. 2. Plasma 5-hydroxytryptamine (5-HT) concentration (*A*) after multidrug treatment with coconut oil (control); bupropion (BUP) and decynium-22 (D-22) (+B +D); or BUP, D-22, and fluoxetin (FLX) (+B +D +FLX) and tissue 5-HT uptake in control fis tissues (*B*). Values are means ± SE. Bars not sharing a letter are significantl different from each other [$P < 0.05$; Kruskal-Wallis test with Dunn's multiple comparisons test (*A*) or one-way ANOVA with Tukey's multiple comparisons test (*B*); $n = 6$].

0.25 h) levels in control fish 42% lower than initial values in +B +D fish and 47% lower than initial values in +B +D +FLX fish [^{3}H] was detected in the anterior water chambers throughout the 16-h experiment (Fig. 4*B*). By 16 h, [^{3}H] in the anterior chambers had increased 5.2-fold from initial ($t = 0.25$ h) levels for control fish 5.3-fold from initial levels for +B +D fish and 8-fold from initial levels for +B +D +FLX fis (Fig. 4*B*). In posterior chambers, [^{3}H] had increased by 20.1-fold for control fis and by 29.5-fold for +B +D fis after 16 h; however, excretion of [^{3}H] did not increase for +B +D +FLX fis (Fig. 4*C*). Over the entire 16-h period, control fis excreted 4.3-fold and +B +D fis 2.6-fold more [^{3}H] per hour into the posterior compartment than into the anterior compartment (Fig. 4*D*). In contrast, there was no difference in overall excretion rate between compartments for +B +D +FLX fish with +B +D +FLX fis excreting 71% less [^{3}H] per hour into the posterior compartment than both control and +B +D fis (Fig. 4*D*). There was a significan interaction between treatment and compartment factors.

DISCUSSION

The pattern of uptake in control fis after the 32-min isotope equilibration period of *series 1* experiments, namely the relatively steady uptake across most tissues, with the greatest per-gram uptake (and the greatest per-tissue uptake) measured in the kidney, suggests that most tissues take up 5-HT from the plasma over this time frame but that most of the 5-HT (and/or its metabolites) is eventually cleared from the body via the kidney. This is consistent with the primarily renal excretion of 5-HT and its metabolites in mammals (57). In contrast, after a shorter 2-min isotope equilibration period in *series 2*, the renal uptake of 5-HT was over twofold greater than after the longer 32-min equilibration period. That uptake was reduced after 32 min of isotope equilibration suggests that significan renal elimination of 5-HT (and/or its metabolites) may be occurring during that time. However, most interesting was that the per-gram uptake in the atrium and ventricle after the 2-min equilibrium period was markedly elevated compared with all other tissues (and that the per-tissue uptake in the ventricle was the second highest among all tissues), suggesting that the heart may be uniquely important for rapid removal of 5-HT from the bloodstream. This findin is consistent with previous reports of high relative SERT mRNA expression in the toadfis heart (30-fold greater than in the brain) (2), apparently high SERT mRNA expression in the goldfis heart (60), and reports of 5-HT immunoreactivity in the atrium of zebrafis (84) and

Table 3. *Whole tissue 5-HT uptake for control fish 7 min after isotope injection in order of increasing uptake*

Tissue	5-HT Uptake, ng/tissue
Cerebellum	0.001 ± 0.000 (6)[a]
Bladder	0.020 ± 0.006 (6)[b]
Brain (–cerebellum)	0.028 ± 0.006 (6)[b]
Gall bladder	0.041 ± 0.009 (6)[b]
Bulbus arteriosus	0.045 ± 0.012 (6)[b,c]
Gonad	0.090 ± 0.040 (6)[b,c,d]
Spleen	0.226 ± 0.064 (6)[c,d,e]
Stomach	0.294 ± 0.055 (6)[d,e,f]
Atrium	1.003 ± 0.412 (6)[e,f,g]
First gill arch	1.153 ± 0.172 (6)[f,g,h]
Third gill arch	1.167 ± 0.193 (6)[f,g,h]
Second gill arch	1.352 ± 0.198 (6)[g,h]
Swim bladder	1.369 ± 0.525 (6)[e,f,g,h]
Intestine	2.061 ± 0.708 (6)[g,h]
Liver	2.346 ± 0.923 (6)[g,h]
Ventricle	2.714 ± 0.636 (6)[g,h]
Kidney	5.430 ± 1.345 (6)[h]

Values are means ± SE (*n*). 5-HT, 5-hydroxytryptamine. Entries not sharing a letter are significantl different ($P < 0.05$; one-way ANOVA with Tukey's multiple comparisons test).

confirm the toadfis heart's ability to sequester 5-HT from the bloodstream.

In contrast to our hypothesis, SERT inhibition by FLX alone did not, at face value, cause a reduction in 5-HT uptake and, in fact, appeared to increase uptake. However, plasma 5-HT was persistently elevated in FLX-treated fish which raised the possibility that the systemic increase in 5-HT created a favorable gradient for 5-HT movement into tissues through non-SERT-mediated pathways. Indeed, when plasma 5-HT was considered, the relative uptake of 5-HT in FLX-treated fis was lower in both the heart and gill, as well as in other tissues, compared with that in control fish The potential involvement of non-SERT pathways is supported by previous studies in mammals showing that the neurotransmitter transporters NET and DAT, as well as various other low-affinit transporters (such as organic cation transporters, extraneuronal monoamine transporters, and the plasma membrane monoamine transporter), can also transport 5-HT, particularly when extracellular 5-HT is elevated or when SERT is inhibited or knocked out (5, 15, 28). That 5-HT uptake per gram of tissue was signifi cantly reduced in the atrium, ventricle, and gill of +B +D +FLX fis compared with control (and in the atrium compared with +B +D fish suggests that, as in mammals, promiscuous or low-affinit transporters do play a role in 5-HT uptake in toadfis under conditions of elevated plasma 5-HT and when SERT is inhibited (5, 15, 28). These experiments also revealed the substantial involvement of SERT in 5-HT uptake, reflectin our relative 5-HT uptake calculations from the FLX-only experiment and supporting our hypothesis that 5-HT uptake in tissues with high SERT mRNA expression, as measured by Amador and McDonald (2), would be at least partly SERT dependent. The SERT-mediated component can be estimated as the difference between uptake in +B +D fis and uptake in +B +D +FLX fish expressed as a percentage of the uptake in +B +D fish in the atrium, ventricle, and gill, SERT may thus be responsible for ~82%, 76%, and 55% of uptake, respectively. Interestingly, the lack of effect of drug treatment in the bulbus suggests that uptake in this tissue is not strongly SERT mediated.

The apparently intermediate effect of +B +D treatment in comparison to control or +B +D +FLX treatment in the atrium, ventricle, and gill, which was significantl different from the effect of +B +D +FLX treatment in the atrium and from the effect of the control treatment in the ventricle, is compelling and suggests that NET, DAT, and/or low-affinit cation transporters may be responsible for a portion of 5-HT uptake under normal physiological conditions in the toadfish This is consistent with finding that, in vitro, a portion of 5-HT uptake into rat synaptosomes is mediated by low-affinit transporters, even at nanomolar concentrations of 5-HT (28), such as those circulating in the toadfish In mammals, low-affinit organic cation transporters have been detected in multiple peripheral tissues, including the heart and lungs (41), and NET is expressed in several tissues, including the lungs (18), suggesting the potential for expression of these transporters in fis cardiac and respiratory tissues. Thus far, NET, DAT, and low-affinit transporters have been identifie in the central nervous system of teleosts (35, 75, 90), but their existence in the periphery, and their potential for promiscuous transport of 5-HT, remains understudied in fish

The ability of the heart and gill to actively remove 5-HT from the bloodstream may be advantageous given the position of these organs within the circulatory system; unlike other organs, the heart and gill encounter the entire blood supply and are thus well suited to tightly regulate circulating 5-HT. Furthermore, the heart may play a particularly important role by removing excess 5-HT from the plasma immediately before it reaches the gill. In fish intra-arterial 5-HT injection can cause vasoconstriction in the gill to the detriment of gas exchange (23, 86). 5-HT uptake by the heart could help prevent unchecked branchial vasoconstriction, and elevated uptake might even contribute to a dilation of gill blood vessels and an increase in branchial gas exchange. Such an important role for the toadfis heart would be consistent with its elevated SERT mRNA expression relative to that of other tissues (2). Interestingly, Caamaño-Tubío et al. (11) proposed that the heart plays little to no role in peripheral 5-HT homeostasis in the rainbow trout; however, the rainbow trout is a hypoxia-intolerant species, and it is currently unknown whether the trout heart shows elevated SERT mRNA expression compared with other tissues. In contrast, toadfis and goldfis show elevated SERT transcript in the heart (2, 60) and are remarkably hypoxia tolerant (33, 49, 51, 92). We suggest that sequestration of 5-HT from the blood by the heart and gill may be an adaptation to help these fis survive in low-oxygen environments. Indeed, acute treatment with FLX attenuates the toadfis hypoxia response, suggesting the potential importance of SERT or the control of circulating 5-HT in tolerating hypoxia (3, 67).

There was minimal uptake of 5-HT from the plasma into the brain, and there was no change in uptake in the brain, even with the +B +D +FLX treatment that resulted in higher plasma 5-HT. That the steady brain uptake across treatments did not match the pattern of 5-HT in the blood indicates that the brain uptake was not simply via passive diffusion. In mammals, the blood-brain barrier is formed by the brain capillary epithelia and serves to physically and functionally separate the composition of the brain microenvironment from fluctuation of solutes within the blood that could alter it (1). The barrier has been reported to be impermeable to neurotransmitters like 5-HT (68); however, Bulat and Supek (9) demonstrated that

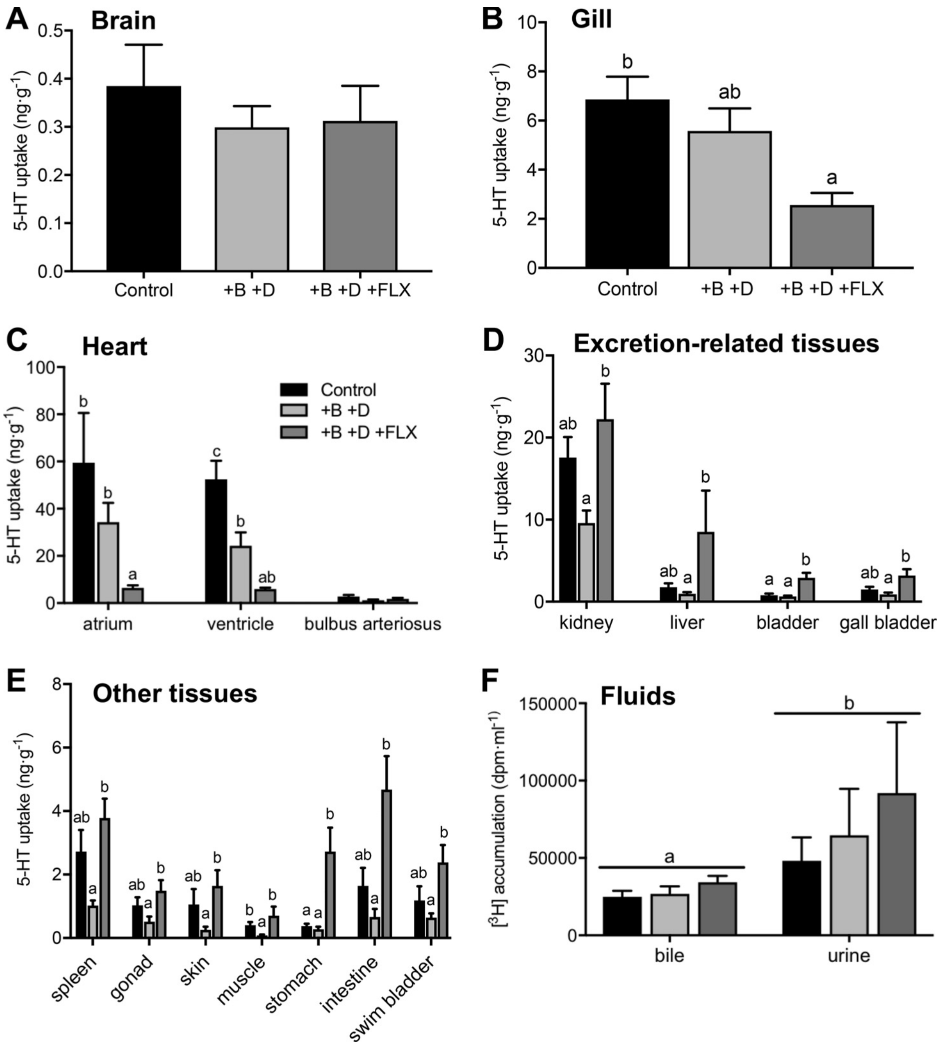

Fig. 3. Uptake of 5-hydroxytryptamine (5-HT) into the brain (*A*), heart (*B*), gill (*C*), excretion-related tissues (*D*), and other tissues (*E*) and accumulation of [^{3}H] (*F*) in bile and urine after multidrug treatment. Values are means ± SE. Bars or groups of bars not sharing a letter within each tissue are significantl different from each other [$P < 0.05$; one-way ANOVA with Tukey's multiple comparisons test (*A*–*E*) or two-way ANOVA (*F*); $n = 6$ except for +B +D +FLX brain ($n = 5$), +B +D and +B +D +FLX urine ($n = 5$), and control urine ($n = 4$)]. +B +D, bupropion and decynium-22; +B +D +FLX, bupropion, decynium-22, and flu oxetine.

injected 5-HT enters the brain dose-dependently through passive diffusion, though brain uptake levels in that study were lower than those in the liver or lung. In teleost fish Genot et al. (25) revealed that the eel brain is at least somewhat permeable to injected 5-HT, accumulating 15%–23.1% as much 5-HT as the liver after 10 min of infusion in vivo and suggesting the presence of at least a partial blood-brain barrier. In the present study, the toadfis brain accumulated 36.1% and 18.4% as much [^{3}H] as the liver after 2 min and 32 min, respectively. A partial blood-brain barrier in toadfis could explain why [^{3}H]5-HT uptake was detectable in the brain but was not readily altered by drug treatment or by the rise in plasma 5-HT. A partial barrier would also be consistent with brain 5-HT uptake being significantl lower than the uptake in most other tissues over the two different time scales, despite the brain expressing similar amounts of SERT mRNA; such a barrier could further explain why uptake was equivalent in the cerebellum and the brain (minus the cerebellum) despite the drastically higher SERT mRNA expression in the toadfis cerebellum than in other brain regions (2).

Table 4. *Accumulation of [^{3}H] in bile, urine, and water under control conditions over 16 h*

	[^{3}H] Accumulation		
Time, h	Bile (× 10^3 cpm/ml)	Urine (× 10^3 cpm/ml)	Water (× 10^3 cpm/g)
0.25	8.3 ± 4.1 (4)	77.6 ± 51.5 (3)	1.3 ± 0.7 (4)
0.5	28.4 ± 16.7 (4)	723.8 ± 543.1 (4)*	4.8 ± 1.5 (4)
1	45.3 ± 24.0 (4)	324.8 ± 171.4 (3)	4.3 ± 3.9 (4)
2	168.8 ± 69.6 (4)†	840.2 ± 377.2 (4)†	5.1 ± 1.9 (4)
4	349.6 ± 177.4 (4)†	1505.5 ± 561.8 (4)†	11.5 ± 4.3 (4)
8	326.9 ± 253.7 (3)†	542.2 ± 210.2 (4)†	9.5 ± 2.3 (4)
16	319.7 ± 108.3 (4)†	523.7 ± 242.4 (4)†	6.5 ± 2.0 (4)

Values are means ±SE (*n*). cpm, counts per minute. Within each flui type, †significan difference from the value at $t = 0.25$ h ($P < 0.05$; one-way ANOVA with Tukey's multiple comparisons test), *significan difference from bile within that time point ($P < 0.05$; Student's *t*-test).

The measurement of 5-HT uptake by the excretory tissues (the kidney, liver, gall bladder, and urinary bladder), especially over the long-term (up to 16 h in the present study), is likely influence by 5-HT metabolism. During 5-HT metabolism, the amino group and an additional hydrogen are firs cleaved by monoamine oxidase to form 5-hydroxyindoleacetaldehyde and ammonia; the transient 5-hydroxyindoleacetaldehyde is then oxidized to form the major 5-HT metabolite, 5-HIAA (76). Alternatively, 5-hydroxyindoleacetaldehyde can be reduced to form 5-hydroxytryptophol, although this is usually a minor

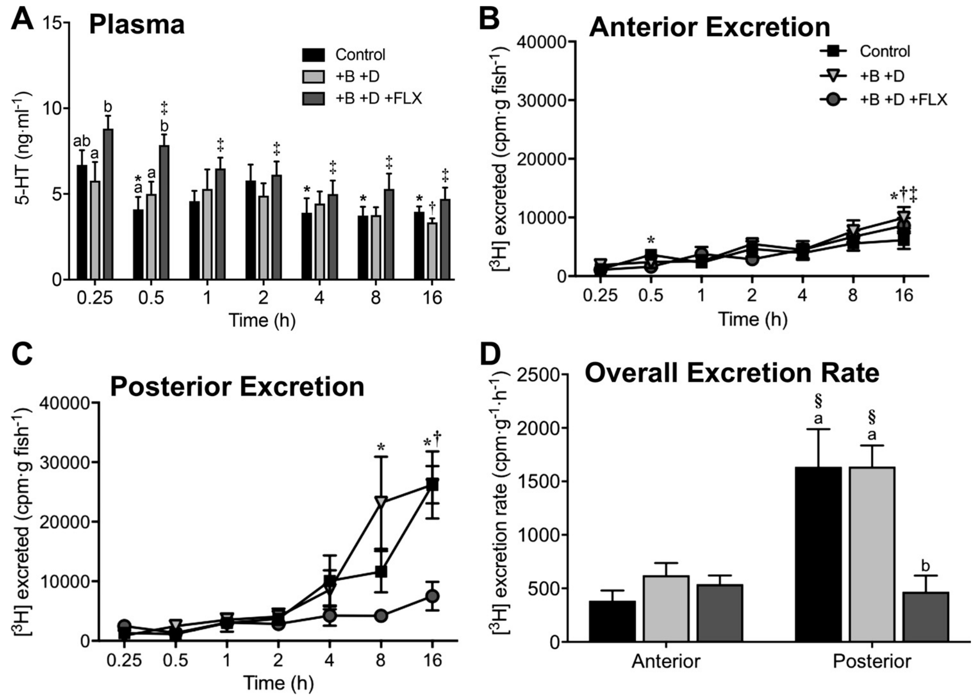

Fig. 4. Time course of plasma 5-hydroxytryptamine (5-HT) over 16 h (*A*), [^{3}H] excretion from 5-HT anterior (*B*) and posterior pathways (*C*), and overall [^{3}H] excretion rates (*D*) after multidrug treatment. Values are means ± SE. Bars in *A* not sharing a letter are significantl different from each other within each time point ($P < 0.05$; repeated measures two-way ANOVA with Tukey's multiple comparisons test; $n = 5$). Significan differences (*A–C*) from initial values are indicated as within the control group (*), within the +B +D group (†), and within the +B +D +FLX group (‡) [$P < 0.05$; repeated measures two-way ANOVA with Dunnett's multiple comparisons test (*A*), repeated measures one-way ANOVA with Dunnett's multiple comparisons test (*B*, control and +B +D +FLX; *C*, +B +D and +B +D +FLX), or Friedman's test with Dunn's multiple comparisons test (*B*, +B +D; *C*, control); $n = 5$]. Within each treatment, §significan difference between compartments (*D*); within a compartment, different lowercase letters denote a significan difference ($P < 0.05$; two-way ANOVA with Sidak's multiple comparisons tests; $n = 5$). +B +D, bupropion and decynium-22; +B +D +FLX, bupropion, decynium-22, and fluoxetine cpm, counts per minute.

metabolic pathway (82). Given that the [^{3}H]5-HT used in this study was tritiated at the 1,2 positions, the main metabolic pathway could ultimately produce one molecule of [^{3}H]5-HIAA, one molecule of [^{3}H]NH_3, and one molecule of [^{3}H]NAD(P)H for each molecule of [^{3}H]5-HT. In rainbow trout plasma, levels of 5-HIAA are approximately twice as high as the levels of 5-HT, likely because of 5-HT metabolism by peripheral tissues and subsequent release into the bloodstream for elimination (11), and plasma total ammonia concentrations in the toadfis are ~3.4 mg/l (74), several orders of magnitude higher than the observed concentrations of 5-HT (in the ng/l range). Thus, we must recognize the possibility that our calculated 5-HT uptake is based in part on radioactivity conferred by the radioactive metabolites of [^{3}H]5-HT rather than by [^{3}H]5-HT alone. This may be particularly the case in excretory tissues and was probably the case for urine, bile, and water (and is the reason the appearance of [^{3}H] instead of 5-HT concentration was reported for these fluids)

Taking this into consideration, the elevated uptake measured in the kidney compared with that in the liver in the short-term experiments (*series 1* and *2*, 2–32 min after isotope injection) is consistent with reports of higher 5-HT levels in the kidney than in the liver of rainbow trout (11). We might also expect such an elevation to be reflecte as a greater accumulation of 5-HT and its metabolites in the urine compared with the bile after longer time periods. This would be in agreement with a study in mammals which found that, within 24 h of intraperitoneal [^{14}C]5-HT injection, 50%–80% of the radioisotope had been excreted in the urine, whereas only 0.5%–5% had been excreted via the intestine (which would include biliary input) (57). However, in the present study, there was not a significan difference between toadfis urine and bile with respect to [^{3}H] accumulation at most (particularly later) time points, suggesting the bile may play a substantial role in 5-HT or 5-HT metabolite excretion in toadfish In fact, there is evidence to suggest that 5-HT levels are greater in the bile than in the urine of toadfis (Amador and McDonald, unpublished observations). An investigation of the levels of 5-HT metabolites in toadfis urine and bile would lend further insight into the relative importance of these excretory pathways.

Interestingly, the 5-HT uptake by the excretory tissues and the accumulation of [^{3}H] into the urine and bile, at least over the acute time frame (*series 2*, 2 min after isotope injection), do not appear to be SERT-mediated. Instead, excretory tissue 5-HT uptake and flui [^{3}H] accumulation mimic the pattern in plasma 5-HT and may simply reflec the level of blood perfusion [e.g., the kidney is the most highly blood-perfused of the organs examined in rainbow trout (7), albacore tuna (95), and channel catfis (80)]. Over the longer incubation period (*series 4*, up to 16 h after isotope injection), the posterior excretion rate of 5-HT and its metabolites, which includes renal and biliary contributions, was significantl greater than the anterior excretion rate. In addition, the reduction in the posterior excretion rate caused by +B +D +FLX treatment (but not by +B +D treatment) may suggest an inhibition of SERT-mediated 5-HT excretion. In mammals, up to 5%–9% of intraperitoneally administered 5-HT has been reported to be excreted unchanged in the urine (57), and 5-HT has been measured in toadfis urine and bile at several times higher concentrations than in the plasma (Amador and McDonald, unpublished observations). If such 5-HT enters the kidney or liver via SERT, SERT inhibition could remove this 5-HT fraction from the urine and bile. Furthermore, there is likely substantial 5-HT metabolite excretion via the kidney [e.g., in trout urine, 5-HIAA levels of 320 ng/ml have been reported (82), whereas plasma levels are only 1.6 ng/ml (11)], and as 5-HT metabolism is intracellular (8, 78) and dependent on transporter-mediated uptake, systemic SERT blockade, such as that occurring in the +B +D +FLX fish would prevent 5-HT metabolism by tissues such as the gill, liver, and intestine. As a consequence, less 5-HIAA would be formed and secreted into the urine (11, 17, 66). Of note, any 5-HT and/or its metabolites present within toadfis urine would likely have entered via transport-mediated secretion, as toadfis kidney tubules are aglomerular and urine is formed primarily by secretion rather than ultrafiltratio (52, 53, 55, 56); however, 5-HIAA, an organic acid, would likely be translocated by an organic anion transport system (29) that would not have been inhibited by the drugs used in this study. It should also be noted that the potential excretory contributions of the skin, particularly since toadfis do not have scales, and the intestinal flui itself (independent of biliary input), the intestine being tissue with the highest peripheral 5-HT concentrations in fis (11), were not determined in the current study and therefore cannot be excluded as a transport-dependent posterior route of excretion.

The accumulation of 5-HT and its metabolites in the anterior chamber may suggest that excretion of these compounds does not occur exclusively via the bile and urine; rather, some excretion may occur across the gills. The anterior radioactivity is unlikely to represent a leak between chambers, as in the leak trial there was no measurable accumulation of isotope in the anterior chambers 16 h after the posterior chambers were spiked with isotope. The increase in anterior excretion at later time points after +B +D and +B +D +FLX treatment may suggest that gill excretion becomes more important as SERT and other transporters are inhibited. However, it is more likely due to the diffusion gradient caused by increased plasma and local 5-HT after SERT inhibition and, potentially, decreases in gill SERT mRNA expression in response to FLX treatment (3). For example, if SERT and other transporters are normally involved in extracting 5-HT from the plasma or extracellular space for metabolism (66) or storage [e.g., in neuroepithelial cells (6, 16, 40, 102)] it is possible that inhibition of these transporters could elevate local 5-HT enough to cause diffusion out of the gill. There have been no previous reports of branchial 5-HT excretion; however, Gulf toadfis are unique in that they excrete urea from the gill in distinct pulses that are mediated by a 5-HT-regulated mechanism (99). It has been suggested that the pulses may contain molecules other than urea, perhaps for the purpose of chemical communication (12a, 24, 42, 81). Thus, it is possible that 5-HT or any of its metabolites could be excreted in this manner. It is also conceivable that diffusion of 5-HT and its main metabolites could occur across the skin, although this seems less likely because of the documented excretory role and markedly greater surface area of the gill. Alternatively, the radioactivity measured in the anterior chamber water may simply be due to branchial excretion of radioactive ammonia (or urea) produced as a byproduct of [^{3}H]5-HT metabolism.

It should be noted that the FLX used in this study would be expected to inhibit SERT on central and peripheral serotonergic neurons in addition to nonneuronal peripheral SERT. The resulting acute increases in synaptic 5-HT may have reduced the firin of 5-HT neurons and increased the activation of postsynaptic receptors, which may have altered various physiological mechanisms in FLX-treated fish given the widespread influenc of 5-HT in the nervous system (65). It is unclear whether interference with serotonergic nerve functioning in this way would have a significan impact on the uptake and excretion of [^{3}H]5-HT that was introduced directly into the bloodstream.

Perspectives and Significance

The results of the current study suggest that SERT-mediated 5-HT uptake, particularly in the heart and gill, may enable tight regulation of circulating 5-HT concentrations in toadfis in the absence of a circulating storage pool like that of mammalian platelets. The results also suggest, for the firs time, to our knowledge, that the contributions of NET, DAT, and/or low-affinit transporters to 5-HT homeostasis in fis may not be trivial. Furthermore, our finding add to the existing evidence supporting a partial blood-brain barrier for 5-HT in fish As previously reported for other teleosts, renal excretion appears to be an important route for the elimination of 5-HT and its metabolites in toadfish although excretion in the bile and across the gills and skin may also be important. SERT also appears to play a considerable role in peripheral 5-HT metabolism by allowing the entry of 5-HT into metabolic tissues. Further studies in which 5-HT and its metabolites are directly measured in the plasma, kidney, liver, urine, and bile of this teleost and others are necessary to truly determine the effects of SERT inhibition on 5-HT metabolism. In addition, research investigating the mechanisms underlying anterior excretion, particularly the possibility of direct 5-HT or 5-HIAA excretion across the gill, is warranted.

ACKNOWLEDGMENTS

We thank Dr. Martin Grosell for productive discussions regarding radiotracer dynamics and Dr. James Happell for the use of his liquid scintillation counter.

GRANTS

This study was funded by a National Science Foundation Grant IOS-1754550 to M. D. McDonald.

DISCLOSURES

No conflict of interest, financia or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.H.B.A. and M.D.M. conceived and designed research; M.H.B.A. performed experiments; M.H.B.A. analyzed data; M.H.B.A. and M.D.M. interpreted results of experiments; M.H.B.A. prepared figures M.H.B.A. drafted manuscript; M.H.B.A. and M.D.M. edited and revised manuscript; M.H.B.A. and M.D.M. approved fina version of manuscript.

REFERENCES

1. **Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ.** Structure and function of the blood-brain barrier. *Neurobiol Dis* 37: 13–25, 2010. doi:10.1016/j.nbd.2009.07.030.

2. **Amador MHB, McDonald MD.** Molecular and functional characterization of the Gulf toadfis serotonin transporter SLC6A4. *J Exp Biol* 221: jeb170928, 2018. doi:10.1242/jeb.170928.
3. **Amador MHB, Schauer KL, McDonald MD.** Does fluoxetin exposure affect hypoxia tolerance in the Gulf toadfish *Opsanus beta? Aquat Toxicol* 199: 55–64, 2018. doi:10.1016/j.aquatox.2018.03.023.
4. **Anderson CR, Campbell G, O'Shea F, Payne M.** The release of neuronal 5-HT from the intestine of a teleost fish *Platycephalus bassensis*. *J Auton Nerv Syst* 33: 239–246, 1991. doi:10.1016/0165-1838(91)90024-W.
5. **Baganz NL, Horton RE, Calderon AS, Owens WA, Munn JL, Watts LT, Koldzic-Zivanovic N, Jeske NA, Koek W, Toney GM, Daws LC.** Organic cation transporter 3: Keeping the brake on extracellular serotonin in serotonin-transporter-deficien mice. *Proc Natl Acad Sci USA* 105: 18976–18981, 2008. doi:10.1073/pnas.0800466105.
6. **Bailly Y, Dunel-Erb S, Laurent P.** The neuroepithelial cells of the fis gill filament indolamine-immunocytochemistry and innervation. *Anat Rec* 233: 143–161, 1992. doi:10.1002/ar.1092330118.
7. **Barron MG, Tarr BD, Hayton WL.** Temperature-dependence of cardiac output and regional blood flo in rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biol* 31: 735–744, 1987. doi:10.1111/j.1095-8649.1987.tb05276.x.
8. **Bogdanski DF, Weissbach H, Udenfriend S.** The distribution of serotonin, 5-hydroxytryptophan decarboxylase, and monoamine oxidase in brain. *J Neurochem* 1: 272–278, 1957. doi:10.1111/j.1471-4159.1957.tb12082.x.
9. **Bulat M, Supek Z.** The penetration of 5-hydroxytryptamine through the blood-brain barrier. *J Neurochem* 14: 265–271, 1967. doi:10.1111/j.1471-4159.1967.tb09523.x.
10. **Burleson ML, Milsom WK.** Cardio-ventilatory control in rainbow trout: II. Refle effects of exogenous neurochemicals. *Respir Physiol* 101: 289–299, 1995. doi:10.1016/0034-5687(95)00029-D.
11. **Caamaño-Tubío RI, Pérez J, Ferreiro S, Aldegunde M.** Peripheral serotonin dynamics in the rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol C Toxicol Pharmacol* 145: 245–255, 2007. doi:10.1016/j.cbpc.2006.12.017.
12. **Cartolano MC, Amador MHB, Tzaneva V, Milsom WK, McDonald MD.** Extrinsic nerves are not involved in branchial 5-HT dynamics or pulsatile urea excretion in Gulf toadfish *Opsanus beta*. *Comp Biochem Physiol A Mol Integr Physiol* 214: 58–65, 2017. doi:10.1016/j.cbpa.2017.08.009.

12a. **Cartolano MC, Tullis-Joyce P, Kubicki K, McDonald MD.** Do Gulf toadfis use pulsatile urea excretion to chemically communicate reproductive status? *Physiol Biochem Zool.* 2018. doi:10.1086/701497.

13. **Cooper BR, Hester TJ, Maxwell RA.** Behavioral and biochemical effects of the antidepressant bupropion (Wellbutrin): evidence for selective blockade of dopamine uptake in vivo. *J Pharmacol Exp Ther* 215: 127–134, 1980.
14. **Côté F, Fligny C, Fromes Y, Mallet J, Vodjdani G.** Recent advances in understanding serotonin regulation of cardiovascular function. *Trends Mol Med* 10: 232–238, 2004. doi:10.1016/j.molmed.2004.03.007.
15. **Daws LC.** Unfaithful neurotransmitter transporters: focus on serotonin uptake and implications for antidepressant efficacy *Pharmacol Ther* 121: 89–99, 2009. doi:10.1016/j.pharmthera.2008.10.004.

15a. **de Pedro N, Pinillos ML, Valenciano AI, Alonso-Bedate M, Delgado MJ.** Inhibitory effect of serotonin on feeding behavior in goldfish involvement of CRF. *Peptides* 19: 505–511, 1998. doi:10.1016/S0196-9781(97)00469-5.

16. **Dunel-Erb S, Bailly Y, Laurent P.** Neuroepithelial cells in fis gill primary lamellae. *J Appl Physiol* 53: 1342–1353, 1982. doi:10.1152/jappl.1982.53.6.1342.
17. **Edwards D, Hall TR, Brown JA.** The characteristics and distribution of monoamine oxidase (MAO) activity in different tissues of the rainbow trout, *Salmo gairdneri*. *Comp Biochem Physiol C Comp Pharmacol* 84: 73–77, 1986. doi:10.1016/0742-8413(86)90167-2.
18. **Eisenhofer G.** The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol Ther* 91: 35–62, 2001. doi:10.1016/S0163-7258(01)00144-9.
19. **El-Merahbi R, Löffler M, Mayer A, Sumara G.** The roles of peripheral serotonin in metabolic homeostasis. *FEBS Lett* 589: 1728–1734, 2015. doi:10.1016/j.febslet.2015.05.054.
20. **Fånge R.** Fish blood cells. In: *Fish Physiology*, edited by Hoar W, Randall D, Farrell A. Cambridge: Academic, 1992, p. 1–54.
21. **Ferriere F, Khan NA, Meyniel JP, Deschaux P.** Characterisation of serotonin transport mechanisms in rainbow trout peripheral blood lymphocytes: role in PHA-induced lymphoproliferation. *Dev Comp Immunol* 23: 37–50, 1999. doi:10.1016/S0145-305X(98)00041-X.
22. **Forster ME, Forster AH, Davison W.** Effects of serotonin, adrenaline and other vasoactive drugs on the branchial blood vessels of the Antarctic fis *Pagothenia borchgrevinki*. *Fish Physiol Biochem* 19: 103–109, 1998. doi:10.1023/A:1007739015634.
23. **Fritsche R, Thomas S, Perry SF.** Effects of serotonin on circulation and respiration in the rainbow trout *Oncorhynchus mykiss*. *J Exp Biol* 73: 59–73, 1992.
24. **Fulton J, LeMoine CMR, Bucking C, Brix KV, Walsh PJ, McDonald MD.** A waterborne chemical cue from Gulf toadfish *Opsanus beta*, prompts pulsatile urea excretion in conspecifics *Physiol Behav* 171: 92–99, 2017. doi:10.1016/j.physbeh.2016.12.037.
25. **Genot G, Morfin R, Peyraud C.** Blood-brain barrier for serotonin in the eel (*Anguilla anguilla* L.). *Comp Biochem Physiol C Comp Pharmacol* 68: 247–250, 1981. doi:10.1016/0306-4492(81)90025-3.
26. **Gershon MD, Tamir H.** Release of endogenous 5-hydroxytryptamine from resting and stimulated enteric neurons. *Neuroscience* 6: 2277–2286, 1981. doi:10.1016/0306-4522(81)90017-8.
27. **Grosell MH, Hogstrand C, Wood CM.** Cu uptake and turnover in both Cu-acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 38: 257–276, 1997. doi:10.1016/S0166-445X(96)00843-0.
28. **Hagan CE, Schenk JO, Neumaier JF.** The contribution of low-affinit transport mechanisms to serotonin clearance in synaptosomes. *Synapse* 65: 1015–1023, 2011. doi:10.1002/syn.20929.
29. **Hakim R, Watrous WM, Fujimoto JM.** The renal tubular transport and metabolism of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the chicken. *J Pharmacol Exp Ther* 175: 749–762, 1970.
30. **Hall FG.** The influenc of varying oxygen tensions upon the rate of oxygen consumption in marine fishes *Am J Physiol* 88: 212–218, 1928. doi:10.1152/ajplegacy.1929.88.2.212.
31. **Handy RD.** Chronic effects of copper exposure versus endocrine toxicity: two sides of the same toxicological process? *Comp Biochem Physiol A Mol Integr Physiol* 135: 25–38, 2003. doi:10.1016/S1095-6433(03)00018-7.
32. **Hirowatari Y, Hara K, Kamihata H, Iwasaka T, Takahashi H.** High-performance liquid chromatographic method with column-switching and post-column reaction for determination of serotonin levels in platelet-poor plasma. *Clin Biochem* 37: 191–197, 2004. doi:10.1016/j.clinbiochem.2003.11.009.
33. **Hochachka PW.** Glucose and acetate metabolism in fish *Can J Biochem Physiol* 39: 1937–1941, 1961. doi:10.1139/o61-216.
34. **Holzer P, Skofitsch G.** Release of endogenous 5-hydroxytryptamine from the myenteric plexus of the guinea-pig isolated small intestine. *Br J Pharmacol* 81: 381–386, 1984. doi:10.1111/j.1476-5381.1984.tb10089.x.
35. **Holzschuh J, Ryu S, Aberger F, Driever W.** Dopamine transporter expression distinguishes dopaminergic neurons from other catecholaminergic neurons in the developing zebrafis embryo. *Mech Dev* 101: 237–243, 2001. doi:10.1016/S0925-4773(01)00287-8.
36. **Hopkins TE, Wood CM, Walsh PJ.** Interactions of cortisol and nitrogen metabolism in the ureogenic gulf toadfis *Opsanus beta*. *J Exp Biol* 198: 2229–2235, 1995.
37. **Ivančič I, Degobbis D.** An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Res* 18: 1143–1147, 1984. doi:10.1016/0043-1354(84)90230-6.
38. **Janvier JJ, Peyraud-Waïtzenegger M, Soulier P.** Effects of serotonin on the cardio-circulatory system of the European eel (*Anguilla anguilla*) in vivo. *J Comp Physiol B* 166: 131–137, 1996. doi:10.1007/BF00301176.
39. **Jonz MG, Fearon IM, Nurse CA.** Neuroepithelial oxygen chemoreceptors of the zebrafis gill. *J Physiol* 560: 737–752, 2004. doi:10.1113/jphysiol.2004.069294.
40. **Jonz MG, Nurse CA.** Neuroepithelial cells and associated innervation of the zebrafis gill: a confocal immunofluorescenc study. *J Comp Neurol* 461: 1–17, 2003. doi:10.1002/cne.10680.
41. **Koepsell H, Lips K, Volk C.** Polyspecifi organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* 24: 1227–1251, 2007. doi:10.1007/s11095-007-9254-z.
42. **Laurent P, Wood CM, Wang Y, Perry SF, Gilmour KM, Part P, Chevalier C, West M, Walsh PJ.** Intracellular vesicular traffickin in

the gill epithelium of urea-excreting fish *Cell Tissue Res* 303: 197–210, 2001. doi:10.1007/s004410000312.
43. **Lim JE, Porteus CS, Bernier NJ.** Serotonin directly stimulates cortisol secretion from the interrenals in goldfish *Gen Comp Endocrinol* 192: 246–255, 2013. doi:10.1016/j.ygcen.2013.08.008.
44. **Mager EM, Medeiros LR, Lange AP, McDonald MD.** The toadfis serotonin 2A (5-HT($_{2A}$)) receptor: molecular characterization and its potential role in urea excretion. *Comp Biochem Physiol A Mol Integr Physiol* 163: 319–326, 2012. doi:10.1016/j.cbpa.2012.07.013.
45. **Marcinkiewcz CA, Devine DP.** Modulation of OCT3 expression by stress, and antidepressant-like activity of decynium-22 in an animal model of depression. *Pharmacol Biochem Behav* 131: 33–41, 2015. doi:10.1016/j.pbb.2015.01.004.
46. **Martín FJ, Míguez JM, Aldegunde M, Atienza G.** Effect of streptozotocin-induced diabetes mellitus on serotonin measures of peripheral tissues in rats. *Life Sci* 56: 51–59, 1995. doi:10.1016/0024-3205(94)00407-J.
47. **Maurer-Spurej E.** Circulating serotonin in vertebrates. *Cell Mol Life Sci* 62: 1881–1889, 2005. doi:10.1007/s00018-005-5149-5.
48. **McDonald MD.** An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish *Comp Biochem Physiol C Toxicol Pharmacol* 197: 19–31, 2017. doi:10.1016/j.cbpc.2017.03.007.
49. **McDonald MD, Gilmour KM, Barimo JF, Frezza PE, Walsh PJ, Perry SF.** Is urea pulsing in toadfis related to environmental O_2 or CO_2 levels? *Comp Biochem Physiol A Mol Integr Physiol* 146: 366–374, 2007. doi:10.1016/j.cbpa.2006.11.003.
50. **McDonald MD, Gilmour KM, Walsh PJ, Perry SF.** Cardiovascular and respiratory reflexe of the gulf toadfis (*Opsanus beta*) during acute hypoxia. *Respir Physiol Neurobiol* 170: 59–66, 2010. doi:10.1016/j.resp.2009.12.012.
51. **McDonald MD, Gonzalez A, Sloman KA.** Higher levels of aggression are observed in socially dominant toadfis treated with the selective serotonin reuptake inhibitor, fluoxetine *Comp Biochem Physiol C Toxicol Pharmacol* 153: 107–112, 2011. doi:10.1016/j.cbpc.2010.09.006.
52. **McDonald MD, Grosell M.** Maintaining osmotic balance with an aglomerular kidney. *Comp Biochem Physiol A Mol Integr Physiol* 143: 447–458, 2006. doi:10.1016/j.cbpa.2005.12.029.
53. **McDonald MD, Grosell M, Wood CM, Walsh PJ.** Branchial and renal handling of urea in the gulf toadfish *Opsanus beta*: the effect of exogenous urea loading. *Comp Biochem Physiol A Mol Integr Physiol* 134: 763–776, 2003. doi:10.1016/S1095-6433(03)00010-2.
54. **McDonald MD, Walsh PJ.** Dogmas and controversies in the handling of nitrogenous wastes: 5-HT$_2$-like receptors are involved in triggering pulsatile urea excretion in the gulf toadfish *Opsanus beta*. *J Exp Biol* 207: 2003–2010, 2004. doi:10.1242/jeb.00957.
55. **McDonald MD, Walsh PJ, Wood CM.** Branchial and renal excretion of urea and urea analogues in the plainfi midshipman, *Porichthys notatus*. *J Comp Physiol B* 172: 699–712, 2002. doi:10.1007/s00360-002-0299-3.
56. **McDonald MD, Wood CM, Wang Y, Walsh PJ.** Differential branchial and renal handling of urea, acetamide and thiourea in the gulf toadfis *Opsanus beta*: evidence for two transporters. *J Exp Biol* 203: 1027–1037, 2000.
57. **McISAAC WM, Page IH.** The metabolism of serotonin (5-hydroxytryptamine). *J Biol Chem* 234: 858–864, 1959.
58. **Medeiros LR, Mager EM, Grosell M, McDonald MD.** The serotonin subtype 1A receptor regulates cortisol secretion in the Gulf toadfish *Opsanus beta*. *Gen Comp Endocrinol* 168: 377–387, 2010. doi:10.1016/j.ygcen.2010.05.004.
59. **Medeiros LR, McDonald MD.** Elevated cortisol inhibits adrenocorticotropic hormone- and serotonin-stimulated cortisol secretion from the interrenal cells of the Gulf toadfis (*Opsanus beta*). *Gen Comp Endocrinol* 179: 414–420, 2012. doi:10.1016/j.ygcen.2012.09.011.
60. **Mennigen JA, Lado WE, Zamora JM, Duarte-Guterman P, Langlois VS, Metcalfe CD, Chang JP, Moon TW, Trudeau VL.** Waterborne fluoxetin disrupts the reproductive axis in sexually mature male goldfish *Carassius auratus*. *Aquat Toxicol* 100: 354–364, 2010. doi:10.1016/j.aquatox.2010.08.016.
61. **Mercado CP, Kilic F.** Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. *Mol Interv* 10: 231–241, 2010. doi:10.1124/mi.10.4.6.
62. **Morando MB, Medeiros LR, McDonald MD.** Fluoxetine treatment affects nitrogen waste excretion and osmoregulation in a marine teleost fish *Aquat Toxicol* 95: 164–171, 2009. doi:10.1016/j.aquatox.2009.10.015.
63. **Nathan FM, Ogawa S, Parhar IS.** Kisspeptin1 modulates odorant-evoked fear response via two serotonin receptor subtypes (5-HT$_{1A}$ and 5-HT$_2$) in zebrafish *J Neurochem* 133: 870–878, 2015. doi:10.1111/jnc.13105.
64. **Nowicki M, Tran S, Muraleetharan A, Markovic S, Gerlai R.** Serotonin antagonists induce anxiolytic and anxiogenic-like behavior in zebrafis in a receptor-subtype dependent manner. *Pharmacol Biochem Behav* 126: 170–180, 2014. doi:10.1016/j.pbb.2014.09.022.
65. **Olivier B.** Serotonin: a never-ending story. *Eur J Pharmacol* 753: 2–18, 2015. doi:10.1016/j.ejphar.2014.10.031.
66. **Olson KR.** Hormone metabolism by the fis gill. *Comp Biochem Physiol A Mol Integr Physiol* 119: 55–65, 1998. doi:10.1016/S1095-6433(97)00406-6.
67. **Panlilio JM, Marin S, Lobl MB, McDonald MD.** Treatment with the selective serotonin reuptake inhibitor, fluoxetine attenuates the fis hypoxia response. *Sci Rep* 6: 31148, 2016. doi:10.1038/srep31148.
68. **Pardridge WM, Oldendorf WH, Cancilla P, Frank HJL.** Blood-brain barrier: interface between internal medicine and the brain. *Ann Intern Med* 105: 82–95, 1986. doi:10.7326/0003-4819-105-1-82.
70. **Pellegrino D, Acierno R, Tota B.** Control of cardiovascular function in the icefis *Chionodraco hamatus*: involvement of serotonin and nitric oxide. *Comp Biochem Physiol A Mol Integr Physiol* 134: 471–480, 2003. doi:10.1016/S1095-6433(02)00324-0.
71. **Prasad P, Ogawa S, Parhar IS.** Role of serotonin in fis reproduction. *Front Neurosci* 9: 195, 2015. doi:10.3389/fnins.2015.00195.
72. **Rahmatullah M, Boyde TR.** Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. *Clin Chim Acta* 107: 3–9, 1980. doi:10.1016/0009-8981(80)90407-6.
73. **Rapport MM.** Serum vasoconstrictor (serotonin) the presence of creatinine in the complex; a proposed structure of the vasoconstrictor principle. *J Biol Chem* 180: 961–969, 1949.
74. **Rodela TM, Esbaugh AJ, Weihrauch D, Veauvy CM, McDonald MD, Gilmour KM, Walsh PJ.** Revisiting the effects of crowding and feeding in the gulf toadfish *Opsanus beta*: the role of Rhesus glycoproteins in nitrogen metabolism and excretion. *J Exp Biol* 215: 301–313, 2012. doi:10.1242/jeb.061879.
75. **Roubert C, Sagné C, Kapsimali M, Vernier P, Bourrat F, Giros B.** A $Na^{(+)}/Cl^{(-)}$-dependent transporter for catecholamines, identifie as a norepinephrine transporter, is expressed in the brain of the teleost fis medaka (*Oryzias latipes*). *Mol Pharmacol* 60: 462–473, 2001.
76. **Ruddell RG, Mann DA, Ramm GA.** The function of serotonin within the liver. *J Hepatol* 48: 666–675, 2008. doi:10.1016/j.jhep.2008.01.006.
77. **Sandblom E, Axelsson M.** Autonomic control of circulation in fish a comparative view. *Auton Neurosci* 165: 127–139, 2011. doi:10.1016/j.autneu.2011.08.006.
78. **Schnaitman C, Greenawalt JW.** Enzymatic properties of the inner and outer membranes of rat liver mitochondria. *J Cell Biol* 38: 158–175, 1968. doi:10.1083/jcb.38.1.158.
79. **Schneider H, Fritzky L, Williams J, Heumann C, Yochum M, Pattar K, Noppert G, Mock V, Hawley E.** Cloning and expression of a zebrafis 5-HT($_{2C}$) receptor gene. *Gene* 502: 108–117, 2012. doi:10.1016/j.gene.2012.03.070.
80. **Schultz IR, Barron MG, Newman MC, Vick AM.** Blood flo distribution and tissue allometry in channel catfish *J Fish Biol* 54: 1275–1286, 1999. doi:10.1111/j.1095-8649.1999.tb02054.x.
81. **Sloman KA, McDonald MD, Barimo JF, Lepage O, Winberg S, Wood CM, Walsh PJ.** Does pulsatile urea excretion serve as a social signal in the gulf toadfis *Opsanus beta? Physiol Biochem Zool* 78: 724–735, 2005. doi:10.1086/432140.
82. **Some M, Helander A.** Urinary excretion patterns of 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophol in various animal species: implications for studies on serotonin metabolism and turnover rate. *Life Sci* 71: 2341–2349, 2002. doi:10.1016/S0024-3205(02)02043-X.
83. **Speeter ME, Heinzelmann RV, Weisblat DI.** The synthesis of the blood serum vasoconstrictor principle serotonin creatinine sulfate. *J Am Chem Soc* 73: 5514–5515, 1951. doi:10.1021/ja01155a580.
84. **Stoyek MR, Jonz MG, Smith FM, Croll RP.** Distribution and chronotropic effects of serotonin in the zebrafis heart. *Auton Neurosci* 206: 43–50, 2017. doi:10.1016/j.autneu.2017.07.004.
85. **Sundin L, Holmgren S, Nilsson S.** The oxygen receptor of the teleost gill? *Acta Zool* 79: 207–214, 1998. doi:10.1111/j.1463-6395.1998.tb01159.x.
86. **Sundin L, Nilsson GE, Block M, Löfman CO.** Control of gill filamen blood flo by serotonin in the rainbow trout, *Oncorhynchus mykiss*. *Am J Physiol Regul Integr Comp Physiol* 268: R1224–R1229, 1995. doi:10.1152/ajpregu.1995.268.5.R1224.

87. **Tavares-Dias M, Oliveira SR.** A review of the blood coagulation system of fish *Rev Rev Bras Biociências Brazilian J Biosci* 4849: 205–224, 2009.
88. **Ultsch GR, Jackson DC, Moalli R.** Metabolic oxygen conformity among lower vertebrates: The toadfis revisited. *J Comp Physiol B* 142: 439–443, 1981. doi:10.1007/BF00688973.
89. **Velarde E, Delgado MJ, Alonso-Gómez AL.** Serotonin-induced contraction in isolated intestine from a teleost fis (*Carassius auratus*): characterization and interactions with melatonin. *Neurogastroenterol Motil* 22: e364–e373, 2010. doi:10.1111/j.1365-2982.2010.01605.x.
90. **Verri T, Terova G, Romano A, Barca A, Pisani P, Storelli C, Saroglia M.** The SoLute Carrier (SLC) family series in teleost fish In: *Functional Genomics in Aquaculture*, edited by Saroglia M, Liu Z. Hoboken, NJ: Wiley, 2012, p. 219–320.
91. **Walsh PJ, Danulat E, Mommsen TP.** Variation in urea excretion in the gulf toadfis Opsanus beta. *Mar Biol* 106: 323–328, 1990. doi:10.1007/BF01344308.
92. **Walsh PJ, Veauvy CM, McDonald MD, Pamenter ME, Buck LT, Wilkie MP.** Piscine insights into comparisons of anoxia tolerance, ammonia toxicity, stroke and hepatic encephalopathy. *Comp Biochem Physiol A Mol Integr Physiol* 147: 332–343, 2007. doi:10.1016/j.cbpa.2006.09.001.
93. **Wang Y, Takai R, Yoshioka H, Shirabe K.** Characterization and expression of serotonin transporter genes in zebrafish *Tohoku J Exp Med* 208: 267–274, 2006. doi:10.1620/tjem.208.267.
94. **Wang Y, Walsh PJ.** High ammonia tolerance in fishe of the family Batrachoididae (Toadfis and Midshipmen). *Aquat Toxicol* 50: 205–219, 2000. doi:10.1016/S0166-445X(99)00101-0.
95. **White FC, Kelly R, Kemper S, Schumacker PT, Gallagher KR, Laurs RM.** Organ blood flo haemodynamics and metabolism of the albacore tuna *Thunnus alalunga* (Bonnaterre). *Exp Biol* 47: 161–169, 1988.
96. **Winberg S, Nilsson G.** Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with special reference to fish *Comp Biochem Physiol* 106C: 597–614, 1993. doi:10.1016/0742-8413(93)90216-8.
97. **Winberg S, Nilsson G.** Time course of changes in brain serotonergic activity and brain tryptophan levels in dominant and subordinate juvenile Arctic charr. *J Exp Biol* 179: 181–195, 1993.
98. **Winberg S, Winberg Y, Fernald RD.** Effect of social rank on brain monoaminergic activity in a cichlid fish *Brain Behav Evol* 49: 230–236, 1997. doi:10.1159/000112994.
99. **Wood C, Hopkins T, Hogstrand C, Walsh P.** Pulsatile urea excretion in the ureagenic toadfis *Opsanus beta*: an analysis of rates and routes. *J Exp Biol* 198: 1729–1741, 1995.
100. **Wood CM, McDonald MD, Sundin L, Laurent P, Walsh PJ.** Pulsatile urea excretion in the gulf toadfish mechanisms and controls. *Comp Biochem Physiol B Biochem Mol Biol* 136: 667–684, 2003. doi:10.1016/S1096-4959(03)00169-6.
101. **Yamaguchi F, Brenner S.** Molecular cloning of 5-hydroxytryptamine (5-HT) type 1 receptor genes from the Japanese puffer fish *Fugu rubripes*. *Gene* 191: 219–223, 1997. doi:10.1016/S0378-1119(97)00064-4.
102. **Zachar PC, Jonz MG.** Neuroepithelial cells of the gill and their role in oxygen sensing. *Respir Physiol Neurobiol* 184: 301–308, 2012. doi:10.1016/j.resp.2012.06.024.