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Serotonergic modulation of zebrafish behavior: Towards a paradox

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ABSTRACT

Due to the fish-specific genome duplication event (~320–350 mya), some genes which code for serotonin proteins were duplicated in teleosts; this duplication event was preceded by a reorganization of the serotonergic system, with the appearance of the raphe nuclei (dependent on the isthmus organizer) and prosencephalic nuclei, including the paraventricular and pretectal complexes. With the appearance of amniotes, duplicated genes were lost, and the serotonergic system was reduced to a more complex raphe system. From a comparative point of view, then, the serotonergic system of zebrafish and that of mammals shows many important differences. However, many different behavioral functions of serotonin, as well as the effects of drugs which affect the serotonergic system, seem to be conserved among species. For example, in both zebrafish and rodents acute serotonin reuptake inhibitors (SSRIs) seem to increase anxiety-like behavior, while chronic SSRIs decrease it; drugs which act at the 5-HT_{1A} receptor seem to decrease anxiety-like behavior in both zebrafish and rodents. In this article, we will expose this paradox, reviewing the chemical neuroanatomy of the zebrafish serotonergic system, followed by an analysis of the role of serotonin in zebrafish fear/anxiety, stress, aggression and the effects of psychedelic drugs.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been proposed to have a plethora of functions in vertebrates, including the control of defensive behavior (Maximino, 2012), the control of sympathetic outflow and the hypothalamus–pituitary–adrenal axis (Lowry, 2002), immunomodulation (Baganz and Blakely, 2013; Khan and Deschaux, 1997), and aggression (Carrillo et al., 2009; Takahashi et al., 2011). These functions have usually been studied largely in mammalian species. With the advent of teleost species, including zebrafish, as important model organisms in the neurosciences (Rinkwitz et al., 2011), however, a paradox begun to shape: while

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, Serotonin, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; 8-OH-DPAT, 7-(Dipropylamino)-5,6,7,8-tetrahydronaphtalen-1-ol; AADC, Aromatic l-amino-acid decarboxylase (EC 4.1.1.28); ACTH, Adrenocorticotropic hormone, corticotropin; AP, Area postrema; *bdnf*, Brain-derived neurotrophic factor; BSF, Blue shortfin wild-type zebrafish; cAMP, 3′,5′-Cyclic adenosine monophosphate; *crh*, *crf*, Corticotropin-releasing hormone; CUS, Chronic unpredictable stress; dpf, Days post-fertilization; DRN, Dorsal raphe nucleus; *etv5b*, ETS variant 5b, erm; fezf2, FEZ family zinc finger 2; *tof*, *fezl*,, Forebrain embryonic zinc finger-like protein 2; GBT, Group behavior task; GC, Griseum centrale, central gray; GR, Glucocorticoid receptor; GR 125,487, 5-Fluoro-2-methoxy-[1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]-1*H*-indole-3-methylcarboxylate sulfamate; Ha, Anterior paraventricular hypothalamus; Hc, Caudal paraventricular hypothalamus; HEK293, Human Embryonic Kidney 293 cells; HEK293-MSR, HEK293 cells expressing the human macrophage scavenger receptor; Hi, Intermediate paraventricular hypothalamus; hpf, Hours post-fertilization; HPI, Hypothalamus–pituitary–interrenal; HSB, High Stationary Behavior zebrafish line; IC_{50}, Half maximal inhibitory concentration; IR, Inferior raphe; K_D, Dissociation constant at equilibrium; K_m, Michaelis–Menten constant; LDT, Light/dark test; LFS, Longfin stripped wild-type zebrafish; *lmx1b*, LIM homeobox transcription factor 1β; LSD, Lysergic acid diethylamide, (6aR,9R)- *N,N*- diethyl- 7-methyl-4,6,6a,7,8,9- hexahydroindolo–[4,3-fg] quinoline- 9-carboxamide; MAO, l-Monoamine oxidase (EC 1.4.3.4); MC-LR, Microcystin-LR; MDMA, 3,4-Methylenedioxy-*N*-methylamphetamine, (*RS*)-1-(benzo[*d*][1,3]dioxol-5-yl)-*N*-methylpropan-2-amine; MiD3cm, Mauthner cell homologue MiD3cm; MK-801, Dizocilpine, [5R,10S]-[+]-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine; MPTP, 1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine; *mr*, Mineralocorticoid receptor; NAN-190, 1-(2-Methoxyphenyl)-4-(4-phthalimidobutyl)piperazine; NE, Norepinephrine; NMDA, *N*-methyl-d-aspartic acid; NOS-1, Nitric oxide synthase isoform 1; *npy*, Neuropeptide Y; NTT, novel tank test, Novel tank diving test; OCT-3, Organic cation transporter 3, extraneuronal monoamine transporter, solute carrier family 22, member 3; OFT, Open-field test; *oxtl*, Oxytocin-like; p.o, *Per os*; PCP, Phenylcyclidine, 1-(1-phenylcyclohexyl)piperidine; pCPA, *para*-Chlorophenylalanine; *pet1*, ETS domain-containing transcription factor 1, FEV; PMAT, Plasma membrane monoamine transporter, equilibrative nucleoside transporter 4, ENT4, solute carrier family 29, member 4; *pomca*, Pro-opiomelanocortin isoform A; *prl2*, Prolactin isoform 2; Rd, Dorsal raphe nucleus; Rm, Medial raphe nucleus; SB 224,289, 1′-Methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4'-piperidinehydrochloride; SERT, Serotonin transporter, 5-HTT, solute carrier family 6 (neurotransmitter transporter), member 4; SIN-1, 3-Morpholinosydnonimine, 5-imino-3-morpholin-4-yl-5*H*-1,2,3-oxadiazol-3-ium-2-ide; SR, Superior raphe; SSRI, Selective serotonin reuptake inhibitor; TH, Tyrosine hydroxylase, tyrosine 3-monooxygenase (EC 1.14.16.2); TPH, Tryptophan hydroxylase, tryptophan 5-monooxygenase (EC 1.14.16.4); *ucn3l*, Urocortin-like isoform 3; UH-301, (S)-5-Fluoro-8-hydroxy-2-(dipropylamino)tetralin; VMAT2, Vesicular monoamine transporter 2, solute carrier family 18 (vesicular monoamine), member 2; WAY 100,635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide; ZBC, Zebrafish Behavior Catalog.

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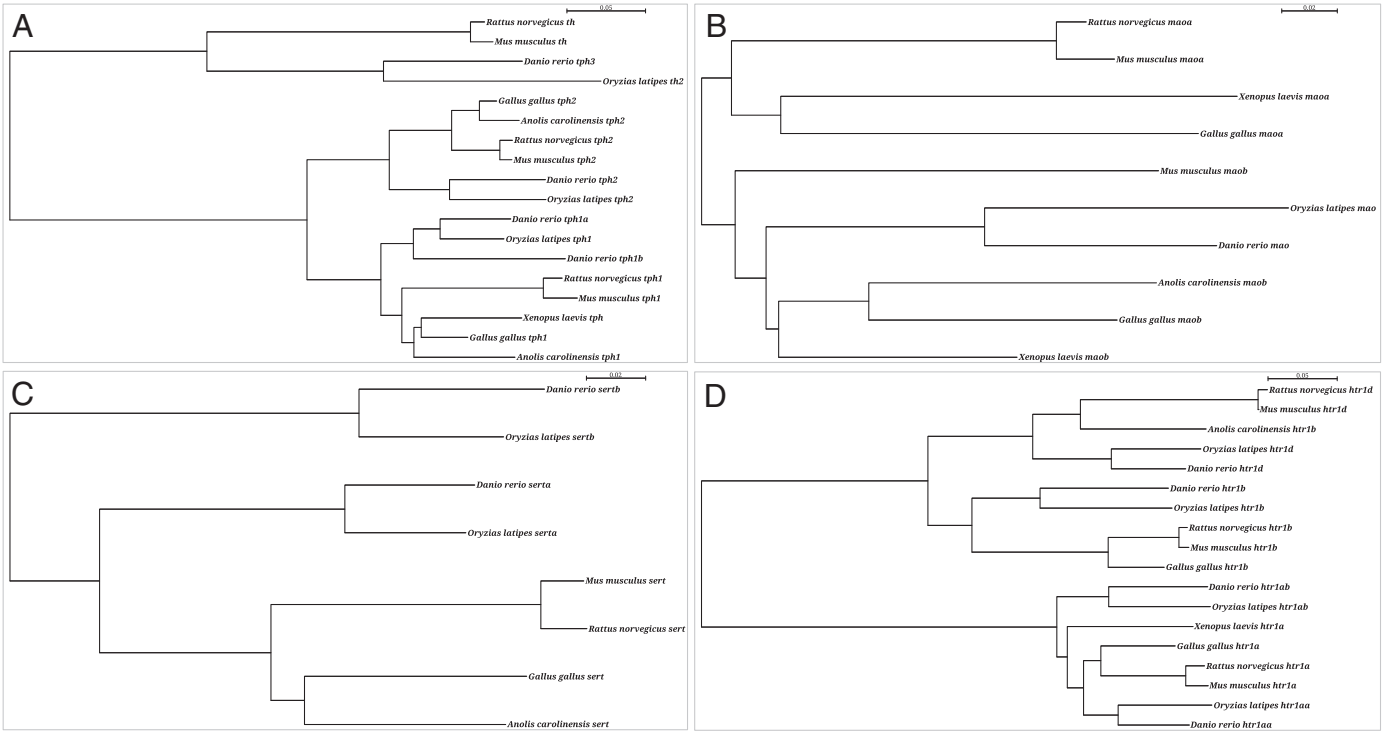

Fig. 1. Phylogenetic trees of selected genes from the serotonergic system in model organisms. (A) Tryptophan hydroxylase; (B) monoamine oxidase; (C) serotonin transporter; (D) 5-HT_{1A} and 5-HT_{1B} receptors. Trees were generated with the Neighbor-Joining with Poisson distances and 100 bootstrapped replicates.

it seems that most of the behavioral functions of serotonin, as well as the effects of drugs which act on that system, seem to be very well conserved, the degree of evolutionary conservation at the genomic and neuroanatomic level is much smaller. In this article, we will expose this paradox, reviewing the chemical neuroanatomy of the zebrafish serotonergic system, followed by an analysis of the role of serotonin in zebrafish fear/anxiety, stress, aggression and the effects of psychedelic drugs.

In order to increase comparability between studies, two strategies were used. First, to facilitate comparison between studies, behavioral variables were accompanied by their code in the Zebrafish Behavior Catalog, v. 1.0 (Kalueff et al., 2013). Second, since in waterborne treatments the unit used to report exposure concentrations varies from molarity to weight per volume, spoiling the comparison of results, all concentrations reported in this article are on the molarity scale.

2. Chemical neuroanatomy of the zebrafish serotonergic system

Early in the ray-finned fish radiation (~320–350 million years ago), prior to or coinciding with the appearance of teleost fishes, a whole-genome duplication event took place, the so-called fish-specific genome duplication (FSGD) or 3R event (Christoffels et al., 2004). Many of the duplicated genes were kept in zebrafish and closely-related teleosts, sometimes termed 'ohnologues' in deference to Ohno (1970), the first proposer of the FSGD. The significance of this event for the evolutionary history of teleosts remains elusive, with some authors proposing the possibility of neofunctionalization (Rastogi and Liberles, 2005), while others propose that the FSGD and subsequent gene loss or differential paralogue evolution in divergent populations can increase speciation (Semon and Wolfe, 2007). Naturally, some genes in the serotonin pathway are duplicated (Fig. 1). In zebrafish, the serotonin transporter and the 5-HT_{1A} receptor present ohnologues (Norton et al., 2008; Wang et al., 2006), while monoamine oxidase has only one isoform (Setini et al., 2005). Tryptophan hydroxylase 1 is duplicated, while tryptophan hydroxylase 2 exists in a single form (Bellipanni et al., 2002; Teraoka et al., 2009). Interestingly, the gene which was previously identified as coding an ohnologue of tyrosine hydroxylase actually encodes for a third tryptophan hydroxylase isoform, albeit its sequence is more similar to that of tyrosine hydroxylase than that of any tryptophan hydroxylase isoform (Ren et al., 2013). That might represent an important example of neofunctionalization of an ohnologue. This lability of the serotonergic system is not exclusive to fishes; an analysis of serotonin genes demonstrated that, while there are no signs of positive or negative selection in rodents and primates (suggesting a functional constraint as the main driving force of the evolution of these genes), considerable heterogeneity in the rate of protein evolution was observed within and between these clades (Andrés et al., 2007).

This duplication event was preceded by a reorganization of the serotonergic system. In the ascidian tunicate tadpole, serotonergic neurons are found only in the hindbrain, while in amphioxus larvae they are found in the forebrain and hindbrain (Candiani et al., 2012); while this situation may resemble that found in Actinopterygii, the existence of forebrain serotonergic nuclei in the amphioxus is probably an apomorphism due to the absence of a midbrain–hindbrain organizer in protochordates (Butler and Hodos, 2005). In the sea lamprey, serotonin-like immunoreactivity is found in the pretectal area, zona limitans intrathalamica, tuberal and mammillary hypothalamus, isthmus and vagal group, as well as in the spinal cord (Barreiro-Iglesias et al., 2009; Cornide-Petronio et al., 2013); these populations are roughly equivalent to the nuclei found in basal actinopterygian fish (López and González, 2014) and teleosts (Lillesaar, 2011; Maximino et al., 2013a). Thus, the ancestral state of the vertebrate serotonergic system is characterized by well-defined nuclei in the raphe nuclei, the preoptic area and the basal hypothalamus (Lillesaar, 2011; López and González, 2014; Maximino et al., 2013a); in amniotes, this system is reduced, as *bona fide* 5-HTergic cells are found only in the retina, pineal and raphe nuclei of these species (Hale and Lowry, 2011).