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Differential gene expression in the brain of *Sebastiscus marmoratus* in response to exposure to polychlorinated biphenyls (PCBs)

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ABSTRACT

Effects of exposure to polychlorinated biphenyls (PCBs) on *Sebastiscus marmoratus* were investigated using a suppression subtractive hybridization method. A total of 108 gene sequences were identified as having the potential for being differentially expressed, and 45 could be identified with homologous database sequences. Functions with which they were associated included long-term potentiation and neurotransmitter release, neuroendocrine, mitosis and cell proliferation, energy-related metabolism, general metabolism, signal protein, hemopoiesis system, immune system, and structure. The expression of 17 of these genes was analyzed in the brain using real time fluorescent quantitative PCR. The present study provided a basis for studying the response of fish to PCB exposure and allowed the characterization of new potential neurotoxicol biomarkers of PCB contamination in seawater.

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of industrial compounds consisting of paired phenyl rings with various degrees of chlorination (Tilson and Kodavanti, 1997). Although their production was banned in the 1970s, this was after more than a billion kilograms were produced, and so they remain ubiquitous, persistent environmental contaminants that are routinely found in samples of human and animal tissues (Fisher, 1999). The central nervous system is one of the target organs for PCBs (Altmann et al., 2001), and PCBs are known to be neurotoxic, inducing neurochemical and behavioral disruptions in humans and experimental animals (Brouwer et al., 1995). Cognitive/motor deficits were found among children who had accidental perinatal exposure to PCBs in cooking oil (Rogan and Gladen, 1992).

It has been proved that disruption of the hypothalamus-pituitary-interrenal axis by PCBs involves brain glucocorticoid receptor down-regulation (Aluru et al., 2004). The effects of PCBs on brain development may be attributable, at least in part, to their ability to reduce circulating levels of thyroid hormone. However, the developmental effects of PCB exposure are not simply a function of PCB-induced hypothyroidism (Zoeller et al., 2000). A high dose of PCBs appears to result in neuronal loss, as reflected by a decrease in glucocorticoid receptor (GR), heat shock protein 90 (hsp90), and heat shock protein 70 (hsp70) expression, leading to the disruption of the negative feedback regulation of cortisol during stress (Aluru et al., 2004). In recent years, learning deficits and endocrine disrupting actions have been observed whereby the non-coplanar congener exhibits neurotoxicities via different mechanisms with coplanar PCB (Zhou et al., 2004).

However, the neurotoxic mechanism of PCBs is complex and actually still unclear. The aim of this study was to identify the transcripts which were up- or down-regulated by PCBs in the brain of the marine fish *Sebastiscus marmoratus* using suppression PCR subtractive hybridization (SSH). This is a highly effective genome-wide approach that enriches for differentially expressed mRNA transcripts by generating subtracted cDNA libraries (Alonso and Leong, 2002; Diatchenko et al., 1996; Straub et al., 2004). Differential expression of some of the genes identified by SSH was confirmed by real time quantitative PCR. This method is very sensitive for the detection and quantification of gene expression levels, in particular for low abundance mRNA (Feng et al., 2007).

2. Materials and methods

2.1. Chemicals

Aroclor 1254, a commercial PCB mixture was purchased from Sigma–Aldrich (Sigma–Aldrich Co., USA), and a PCR-Select cDNA

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Table 1
Characterisation of ESTs isolated from polychlorinated biphenyls-treated *Sebastiscus marmoratus*

Homologous gene and species	EST #	Insert (bp)	*E*-value	Change	GenBank Accession No.
LTP and neurotransmitter release related					
Danio rerio SNAP 25a	9	271	$7e^{-47}$	↓	FF403915
Carassius auratus kainate receptor (AMPA)beta subunit	13	384	$4e^{-63}$	↓	FF403841
O. latipes calmodulin	14	541	$2e^{-162}$	↓	FF403842
Scophthalmus maximus clone sba441 ependymin	20	486	$2e^{-31}$	↓	FF403849
Neuroendocrine					
Gasterosteus aculeatus RTN1	8	321	$7e^{-112}$	↓	FF403914
Paralichthys olivaceus carboxypeptidase H	22	587	$2e^{-132}$	↓	FF403851
Mitosis and Cell proliferation related					
Gillichthys mirabilis transducer of ERBB-2 (Tob)	1	305	$4e^{-30}$	↑	FF403837
Danio rerio dynein cytoplasmic 1 heavy chain 1	6	470	$5e^{-25}$	↓	FF403892
Oreochromis mossambicus stathmin-like mRNA	23	745	0.0	↓	FF403852
Energy-related metabolism					
Scombridae gen. sp. cytochrome *c* oxidase subunit VI b precursor	4	268	$5e^{-60}$	↑	FF403870
Sebastes maliger cytochrome *b*	5	326	$1e^{-91}$	↑	FF403881
Rhabdosargus sarba sodium potassium ATPase α subunit	15	754	0.0	↓	FF403843
Helicolenus hilgendorfi ATPase 6	26	165	$2e^{-52}$	↑	FF403855
General metabolism					
Danio rerio proteasome subunit Y	32	246	$5e^{-32}$	↓	FF403862
Sphyraena argentea lactate dehydrogenase-A	52	487	$3e^{-47}$	↑	FF403884
Danio rerio glutamic-oxaloacetic transaminase 1, soluble	56	439	$8e^{-92}$	↓	FF403888
Signal proteins					
Danio rerio zinc finger protein zic1	10	379	$4e^{-107}$	↓	FF403838
Sparus aurata V-Fos transformation effector-like protein	11	468	0.0	↓	FF403839
Danio rerio suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	21	687	$5e^{-122}$	↓	FF403850
Siniperca chuatsi clone C311 eukaryotic translation elongation factor -1 gamma	34	270	$5e^{-63}$	↑	FF403864
Danio rerio zinc finger protein zic1	36	197	$1e^{-41}$	↓	FF403866
Homo sapiens zinc finger protein 238 (ZNF238), transcript variant 1	74	310	$1e^{-18}$	↓	FF403908
Immune system					
Epinephelus akaara clone 004 MHC class I alpha antigen	25	353	$5e^{-11}$	↑	FF403854
Hemopoiesis system					
Oryzias latipes embryonic alpha-type globin	12	371	$3e^{-59}$	↓	FF403840
Ribosomal proteins					
Platichthys flesus 60S ribosomal protein L28	24	308	$1e^{-125}$	↑	FF403853
Paralichthys olivaceus ribosomal protein L17	28	456	$1e^{-145}$	↑	FF403857
Siniperca chuatsi clone C426 60S ribosomal protein L35	29	180	$6e^{-71}$	↑	FF403858
Pagrus major 60S ribosomal protein L24	30	346	$6e^{-88}$	↓	FF403860
Solea senegalensis ribosomal protein L35a	51	394	$3e^{-122}$	↑	FF403883
Siniperca chuatsi clone C123 ribosomal protein L7	57	561	0.0	↓	FF403889
Pagrus major ribosomal protein L37	65	259	$2e^{-76}$	↑	FF403898
Identified genes but function unknown					
Tetraodon nigroviridis full-length cDNA	7	428	$1e^{-61}$	↓	FF403903
Tetraodon nigroviridis full-length cDNA	16	342	$3e^{-25}$	↓	FF403844
Gasterosteus aculeatus clone CNB134-E09 mRNA	17	467	$1e^{-52}$	↓	FF403845
Tetraodon nigroviridis full-length cDNA	18	262	$6e^{-21}$	↓	FF403846
Tetraodon nigroviridis full-length cDNA	19	300	$2e^{-16}$	↓	FF403847
Tetraodon nigroviridis full-length cDNA	27	410	$4e^{-127}$	↑	FF403856
Gasterosteus aculeatus clone CFW195-D 03 mRNA	33	220	$1e^{-7}$	↑	FF403863
Tetraodon nigroviridis full-length cDNA	38	474	$2e^{-10}$	↑	FF403868
Tetraodon nigroviridis full-length cDNA	49	111	$3e^{-10}$	↑	FF403880
Gasterosteus aculeatus clone CGX01-B09 mRNA	54	514	$3e^{-60}$	↓	FF403886
Gasterosteus aculeatus clone CNB24-E09 mRNA	62	335	$7e^{-09}$	↑	FF403895
Tetraodon nigroviridis full-length cDNA	73	453	$3e^{-15}$	↑	FF403907
Gasterosteus aculeatus clone CNB134-E09 mRNA	78	264	$5e^{-29}$	↓	FF403912
Unidentified genes					
No homologous region	2	423	–	↑	FF403848
No homologous region	3	453	–	↑	FF403859
No homologous region	35	392	–	↑	FF403865
No homologous region	37	191	–	↑	FF403867
No homologous region	39	328	–	↑	FF403869
No homologous region	40	548	–	↑	FF403871
No homologous region	41	424	–	↓	FF403872
No homologous region	42	496	–	↓	FF403873
No homologous region	43	627	–	↓	FF403874
No homologous region	44	267	–	↓	FF403875
No homologous region	45	595	–	↓	FF403876
No homologous region	46	756	–	↓	FF403877
No homologous region	47	767	–	↓	FF403878
No homologous region	48	611	–	↓	FF403879
No homologous region	50	179	–	↑	FF403882
No homologous region	53	1143	–	↑	FF403885

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