HHS Public Access

Author manuscript

Sustain Chem Pharm. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Sustain Chem Pharm. 2019 September ; 13: . doi:10.1016/j.scp.2019.100148.

The potential association of psychoactive pharmaceuticals in the environment with human neurological disorders

Gaurav Kaushik[1,2,#,§], **Michael A. Thomas**[1]

[1]Department of Biological Sciences, Idaho State University, Stop 8007, 921 S 8th Ave, Pocatello, ID 83209-8007, USA

[2]Stem Pharm, Incorporated, Madison, WI 53711 USA

Abstract

Psychoactive pharmaceuticals release into the environment and reach humans through a variety of routes, including sewage, drinking water, contaminated irrigation water, biosolids, soil and food. It was assumed that these compounds via the environment could induce genetic effects in the etiology of human neurological disorders. With the help of *in vitro*, *in vivo* and *in silico* approaches, we demonstrated that psychoactive pharmaceuticals in drinking water can cross maternal biological barriers and alter *in vitro* molecular and genetic mechanisms that potentially have a key role in the development, growth and regulation of neuronal systems during embryonic brain development.

Keywords

Psychoactive pharmaceuticals; Fluoxetine; Venlafaxine; Carbamazepine; Drinking water; Environmental toxicology

Introduction

Psychoactive pharmaceuticals (especially SSRIs, SNRIs and AEDs) are one of the mostly widely prescribed classes of drugs in the United States. Excretions from clinical patients contain active metabolites and isoforms with long half-lives (Calisto and Esteves, 2009; Thomas and Klaper, 2012). Sewage treatment plants (STP) release treated water containing pharmaceuticals into rivers and lakes, thus introducing contamination into aquatic systems. Due to the excretion of pharmaceuticals and their metabolites by humans and their inefficient removal by STP, these pharmaceuticals might reach potable water (Calisto and Esteves, 2009; Thomas and Klaper, 2012). By reusing sludge as biosolids and by irrigation with polluted surface and groundwater, these contaminants end up in the soil and accumulate in food crops (Goldstein et al., 2014; Riemenschneider et al., 2017). SSRI antidepressants

§ Corresponding author kausgaur@isu.edu.
# Current address

including their metabolites tend to accumulate in brain tissues of fish (Arnnok et al., 2017; Brooks et al., 2005; Lajeunesse et al., 2011; Schultz et al., 2010; Silva et al., 2015; Valdés et al., 2016), other food organisms like mussels (Bringolf et al., 2010; Silva et al., 2016), and in food crops (Goldstein et al., 2014; Malchi et al., 2014; Riemenschneider et al., 2017). Previous laboratory studies demonstrated that gene sets associated with neuronal systems were up- and down-regulated in the brains of fathead minnows treated with psychoactive pharmaceuticals (fluoxetine, venlafaxine and carbamazepine) at low concentrations (Thomas et al., 2012; Thomas and Klaper, 2012). We hypothesized that exposure to psychoactive pharmaceuticals via environment would induce genetic effects associated with human neurological disorders including autism. Using *in vitro*, *in silico*, and *in vivo* approaches, we determined the extent to which psychoactive pharmaceuticals induced molecular patterns similar to neurological disorders (Kaushik et al., 2017, 2016a, 2016b, 2015).

Methods

We cultured human neuronal SK-N-SH cells on a dish and differentiated them into pure neuronal cells with retinoic acid (3 μg/L) for two weeks. We then treated them with a mixture of psychoactive pharmaceuticals (fluoxetine 10μg/L, venlafaxine 50μg/L and carbamazepine 100μg/L) for 48 hrs. We extracted the RNA of treated samples and used them for the RNA sequencing (Kaushik et al., 2016b). In parallel, we analyzed the expression of key synaptic proteins using flow cytometry (Kaushik et al., 2017). With the help of the GAGE enrichment analysis tool, we determined if any neuronal gene set was significantly up- and down-regulated under the treatments (Kaushik et al., 2016b). We then created an autism-associated protein-protein interaction network using Cytoscape and analyzed interactions of induced genes (or, proteins) (Kaushik et al., 2015). To determine the extent to which these pharmaceuticals can cross maternal biological barriers, we added ^{2}H isotope labeled pharmaceuticals into the drinking water of female mice *in vivo,* and measured the $\delta^{2}H$ isotope signal in maternal liver and embryonic brains (Kaushik et al., 2016a).

Results

In Table 1, RNA-sequencing results from *in vitro* study showed that psychoactive pharmaceuticals in a mixture treatment altered the expression of neurological gene sets significantly. Those gene sets were associated with human neurological disorders including Idiopathic Autism, Alzheimer’s disease and Schizophrenia (Kaushik et al., 2016b). Network analysis showed that genes in human cell cultures enriched by pharmaceutical mixtures and valproate (which was used as a positive control at clinical dosages) had similar network signatures and greater connectedness than genes in the overall network (Kaushik et al., 2015). In addition, we found that the expression of autism-associated synaptic proteins (HTR1B, HTR2C, OXTR, NMDAR1, GABRB3, PSD95 and SV2A) were altered significantly in cultured human neuronal cells by psychoactive pharmaceuticals (Kaushik et al., 2017). After analyzing mouse maternal liver and embryonic brains with mass spectrometry, we observed that carbamazepine presented in the maternal drinking water crossed intestinal and placental barriers and reached developing embryonic brains (Kaushik et al., 2016a).

Discussion

This study examined the potential association of psychoactive pharmaceuticals (FLX, CBZ and VNX) with neurological disorders. We hypothesized that psychoactive pharmaceuticals from drinking water and food can cross maternal biological barriers and alter *in vitro* neuronal protein and gene expression associated with neurological disorders. To address this overarching hypothesis, we determined the extent to which psychoactive pharmaceuticals can induce the gene or protein expression patterns associated with neurological disorders in human neuronal cells. The information presented in previous literature concerning the role of psychoactive pharmaceuticals in the etiology of neurological disorders is very limited. Therefore, this study sought to address the issue with different approaches to improve upon limited knowledge of these chemical contaminants present in the environment.

In our findings, we showed that these pharmaceuticals can induce *in vitro* expression of synaptic proteins and neuronal genes that are associated with neurological disorders like Autism, Alzheimer's disease and others (Kaushik et al., 2017, 2016b). We also found that the drug-induced gene expression changes in human neuronal cells represent higher connectedness in an ASD-associated protein-protein interaction network (Kaushik et al., 2015). Due to higher connectedness, the altered gene expression would further peturb more downstream connected proteins, thus creating profound effects in ASD-associated network because of the ripple effect. In addition, we also demonstrated that carbamazepine present in the drinking water can cross maternal intestinal and placental barriers and reached developing embryonic brains (Kaushik et al., 2016a).

Our findings are innovative and significant because they considered the ways in which psychoactive pharmaceuticals altered fundamental molecular expressions that may result in abnormal neuronal growth, regulation, and development, which are implicated in the etiology of human neurological disorders including idiopathic autism. To date, several studies have shown that mutations in candidate genes and other stochastic factors are responsible for only a few cases of idiopathic autism (Gompers et al., 2017, 2016; Kaushik and Zarbalis, 2016; Napoli et al., 2018), but the majority of cases result from the presence of an unknown environmental trigger (especially in the first trimester of pregnancy) in genetically susceptible individuals (Landrigan, 2010).

In the perspective of the bioaccumulation potential of the tested drugs and especially of their metabolites, it may be considered in the future to adjust the concentrations of the model substances. Given the lower BAC/BCF values of carbamazepine and fluoxetine, the test concentration of carbamazepine could be lowered, and the test concentration of fluoxetine can therefore be increased to make the test concentrations more environmentally relevant. Since the metabolites accumulate more strongly and can be more toxic, testing with metabolites may also be considered. Some researchers have also observed the strong accumulation of sertraline and in particular, norsertraline in the brain tissues (Brooks et al., 2005; Schultz et al., 2010), this drug could be a good candidate for the follow-up testing.

Other future studies would include performing mice studies using pharmaceuticals (carbamazepine, fluoxetine and venlafaxine) labeled with different isotopes at different

isotopic concentrations (10%, 20%, 50% and 100%) to understand the transfer rate when those drugs are administered individually and, in a mixture to pregnant mice' drinking water and derive a mathematical relationship. And, in the continuation, it would be useful to further analyze newly born pups behavior and brain samples for in-depth understanding of association of pharmaceuticals at environmental relevant concentrations with human neurological disorders. In addition, many researchers have been creating embryonic brain-mimic models (also known as organoids) using human induced pluripotent stem cells (iPSCs) derived neurons, endothelial cells and microglia (Schwartz et al., 2015). With the help of recently developed tools such as label-free imaging and machine learning, these brain-mimic models can be treated with different neurotoxins at environment-relevant concentrations for high-throughput toxicity screening (Kaushik et al., 2019a, 2019b; Schwartz et al., 2015). The outcomes for these studies would provide more insights into altered genetic effects by environmental contaminants.

Conclusions

As a broader perspective, the current study suggests that psychoactive pharmaceuticals can interact with genetic factors causing altered neuronal growth, regulation and development associated with human neurological disorders.

Acknowledgements

The authors gratefully acknowledge the contribution of the late Prof. Chris Cretekos and thank him for supporting and providing methodological and intellectual expertise to the present study, and we dedicate this study to his memory. We also thank Ms. Shesha Chaturvedi for helping us in creating the abstract image. The project was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant # P20GM103408.

References:

Arnnok P, Singh RR, Burakham R, Pérez-Fuentetaja A, Aga DS, 2017 Selective Uptake and Bioaccumulation of Antidepressants in Fish from Effluent-Impacted Niagara River. Environ. Sci. Technol 10.1021/acs.est.7b02912

Bringolf RB, Heltsley RM, Newton TJ, Eads CB, Fraley SJ, Shea D, Cope WG, 2010 Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. Environ. Toxicol. Chem 10.1002/etc.157

Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, Lewis RJ, 2005 Determination of select antidepressants in fish from an effluent-dominated stream. Environ. Toxicol. Chem

Calisto V, Esteves VI, 2009 Psychiatric pharmaceuticals in the environment. Chemosphere. 10.1016/j.chemosphere.2009.09.021

Goldstein M, Shenker M, Chefetz B, 2014 Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. Environ. Sci. Technol 10.1021/es5008615

Gompers AL, Su-Feher L, Ellegood J, Copping NA, Riyadh MA, Stradleigh TW, Pride MC, Schaffler MD, Wade AA, Catta-Preta R, Zdilar I, Louis S, Kaushik G, Mannion BJ, Plajzer-Frick I, Afzal V, Visel A, Pennacchio LA, Dickel DE, Lerch JP, Crawley JN, Zarbalis KS, Silverman JL, Nord AS, 2017 Germline Chd8 haploinsufficiency alters brain development in mouse. Nat. Neurosci 20, 1062–1073. 10.1038/nn.4592 [PubMed: 28671691]

Gompers AL, Su-Feher L, Ellegood J, Stradleigh TW, Zdilar I, Copping NA, Pride MC, Schaffler MD, Riyadh MA, Kaushik G, Mannion B, Plajzer-Frick I, Afzal V, Visel A, Pennacchio LA, Dickel D, Lerch JP, Crawley JN, Zarbalis KS, Silverman JL, Nord AS, 2016 Heterozygous mutation to

Chd8 causes macrocephaly and widespread alteration of neurodevelopmental transcriptional networks in mouse. bioRxiv 76158 10.1101/076158

Kaushik G, Gil DA, Torr E, Berge ES, Soref C, Uhl P, Fontana G, Antosiewicz-Bourget J, Edington C, Schwartz MP, Griffith LG, Thomson JA, Skala MC, Daly WT, Murphy WL, 2019a Quantitative Label-Free Imaging of 3D Vascular Networks Self-Assembled in Synthetic Hydrogels. Adv. Healthc. Mater 10.1002/adhm.201801186

Kaushik G, Gil DA, Torr E, Berge ES, Soref C, Uhl P, Fontana G, Antosiewicz-Bourget J, Edington C, Schwartz MP, Griffith LG, Thomson JA, Skala MC, Daly WT, Murphy WL, 2019b Vascular Networks: Quantitative Label-Free Imaging of 3D Vascular Networks Self-Assembled in Synthetic Hydrogels (Adv. Healthcare Mater. 2/2019). Adv. Healthc. Mater 8, 1970004 10.1002/adhm.201970004

Kaushik G, Huber DP, Aho K, Finney B, Bearden S, Zarbalis KS, Thomas MA, 2016a Maternal exposure to carbamazepine at environmental concentrations can cross intestinal and placental barriers. Biochem. Biophys. Res. Commun 10.1016/j.bbrc.2016.04.088

Kaushik G, Thomas MA, Aho KA, 2015 Psychoactive pharmaceuticals as environmental contaminants may disrupt highly inter-connected nodes in an Autism-associated protein-protein interaction network. BMC Bioinformatics 16 Suppl 7, S3 10.1186/1471-2105-16-S7-S3

Kaushik G, Xia Y, Pfau JC, Thomas MA, 2017 Dysregulation of autism-associated synaptic proteins by psychoactive pharmaceuticals at environmental concentrations. Neurosci. Lett 10.1016/j.neulet.2017.09.058

Kaushik G, Xia Y, Yang L, Thomas MA, 2016b Psychoactive pharmaceuticals at environmental concentrations induce in vitro gene expression associated with neurological disorders. BMC Genomics 17 10.1186/s12864-016-2784-1

Kaushik G, Zarbalis KS, 2016 Prenatal Neurogenesis in Autism Spectrum Disorders. Front. Chem 10.3389/fchem.2016.00012

Lajeunesse A, Gagnon C, Gagné F, Louis S, Čejka P, Sauvé S, 2011 Distribution of antidepressants and their metabolites in brook trout exposed to municipal wastewaters before and after ozone treatment - Evidence of biological effects. Chemosphere. 10.1016/j.chemosphere.2010.12.026

Landrigan PJ, 2010 What causes autism? Exploring the environmental contribution. Curr. Opin. Pediatr 10.1097/MOP.0b013e328336eb9a

Malchi T, Maor Y, Tadmor G, Shenker M, Chefetz B, 2014 Irrigation of root vegetables with treated wastewater: Evaluating uptake of pharmaceuticals and the associated human health risks. Environ. Sci. Technol 10.1021/es5017894

Napoli E, Song G, Panoutsopoulos A, Riyadh MA, Kaushik G, Halmai J, Levenson R, Zarbalis KS, Giulivi C, 2018 Beyond autophagy: a novel role for autism-linked Wdfy3 in brain mitophagy. Sci. Rep 10.1038/s41598-018-29421-7

Riemenschneider C, Seiwert B, Goldstein M, Al-Raggad M, Salameh E, Chefetz B, Reemtsma T, 2017 An LC-MS/MS method for the determination of 28 polar environmental contaminants and metabolites in vegetables irrigated with treated municipal wastewater. Anal. Methods 9, 1273–1281. 10.1039/C6AY02984A

Schultz MM, Furlong ET, Kolpin DW, Werner SL, Schoenfuss HL, Barber LB, Blazer VS, Norris DO, Vajda AM, 2010 Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: Occurrence and fate in water and sediment and selective uptake in fish neural tissue. Environ. Sci. Technol 10.1021/es9022706

Schwartz MP, Hou Z, Propson NE, Zhang J, Engstrom CJ, Costa VS, Jiang P, Nguyen BK, Bolin JM, Daly W, Wang Y, Stewart R, Page CD, Murphy WL, Thomson JA, 2015 Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. Proc. Natl. Acad. Sci 10.1073/pnas.1516645112

Silva LJG, Martins MC, Pereira AMPT, Meisel LM, Gonzalez-Rey M, Bebianno MJ, Lino CM, Pena A, 2016 Uptake, accumulation and metabolization of the antidepressant fluoxetine by Mytilus galloprovincialis. Environ. Pollut 10.1016/j.envpol.2016.02.022

Silva LJG, Pereira AMPT, Meisel LM, Lino CM, Pena A, 2015 Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: Uptake, bioaccumulation and ecotoxicology. Environ. Pollut 10.1016/j.envpol.2014.12.002

Thomas MA, Joshi PP, Klaper RD, 2012 Gene-class analysis of expression patterns induced by psychoactive pharmaceutical exposure in fathead minnow (Pimephales promelas) indicates induction of neuronal systems, in: Comparative Biochemistry and Physiology - C Toxicology and Pharmacology. 10.1016/j.cbpc.2011.05.014

Thomas MA, Klaper RD, 2012 Psychoactive pharmaceuticals induce fish gene expression profiles associated with human idiopathic autism. PLoS One. 10.1371/journal.pone.0032917

Valdés ME, Huerta B, Wunderlin DA, Bistoni MA, Barceló D, Rodriguez-Mozaz S, 2016 Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals in fish under field and controlled laboratory experiments. Evidences of carbamazepine metabolization by fish. Sci. Total Environ 10.1016/j.scitotenv.2016.03.045

Highlights

- Psychoactive pharmaceuticals have been detected at low concentrations in wastewater, streams and drinking water.
- Psychoactive pharmaceuticals at low concentrations could cross maternal barriers and alter neuronal gene sets significantly during embryonic brain development.
- Altered gene sets are associated with human neurological disorders.

Table 1:

Enrichment of human neurological gene sets

Source	Neurological Disorders gene set (previously published)	Size	Function/Description	Significant change in the expression of the gene set	Significance level
GSE12457	**ADHD_down**	32	Down-regulated genes by molecular alterations in genetic and environmental rat models of ADHD	**Down-regulated**	*P*-value = 0.000 *Q*-value = 0.003
GSE12457	**ADHD_up**	44	Up-regulated genes by molecular alterations in genetic and environmental rat models of ADHD	**Down-regulated**	*P*-value = 0.007 *Q*-value = 0.022
GSE1297	**Alzheimers**	308	Genes upregulated in the CA1 region of the hippocampus in Alzheimer's disease	**Up-regulated**	*P*-value = 0.004 *Q*-value = 0.027
	Autism_idiopathic	439	Combination of Chakrabarti, Hu and ASD_2class; excluding duplicates	**Up-regulated**	*P*-value = 0.000 *Q*-value = 0.001
	Parkinsons	130	Genes associated with Parkinsons	**Down-regulated**	*P*-value = 0.000 *Q*-value = 0.000
	Schizophrenia	29	Differentially expressed proteins in the brains of SCZ patients	**Down-regulated**	*P*-value = 0.000 *Q*-value = 0.000
GSE15402	**ASD_2Class**	354	Differentially expressed genes (significantly) from a SAM 2-class analysis of the data from combined autistic samples and neurotypical controls	**Up-regulated**	*P*-value = 0.000 *Q*-value = 0.000
GSE15402	**ASD_Mild**	306	Differentially expressed genes (significantly) from a SAM 2-class analysis of the data from mild autistic samples and neurotypical controls	**Up-regulated**	*P*-value = 0.000 *Q*-value = 0.000

The table represents the analyses of gene sets associated with neurological disorders. Source indicates the database from where the gene set was derived, Gene Ontology (GO) or Molecular Signatures Database (MSigDB). Size represents the number of genes in each gene set. Significant change indicates the expression of gene sets which were significantly found up-regulated and down-regulated in the mixture treatment of pharmaceuticals on cultured human neuronal cells. Gene sets with *P*-value < 0.01 and *Q*-value < 0.1 were considered as statistically significantly enriched.