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Full Length Research Paper

"Environmental ritalinization": Brain structural changes after exposition to methylphenidate residues

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Methylphenidate, marketed as Ritalin, is an emerged pollutant found in wastewater. Methylphenidate exposure at pharmacological doses can modify brain metabolism, however, the consequences of environmental residues remains to be elucidated. This work presents the results of a chronic exposure to methylphenidate residual levels (0.1875 and 1.875 µg/L) consistent with the amount found in the environment. Animals were exposed to contaminated water during childhood and adolescence. Results evidence significant changes in brain architecture such as decreased cortical and increased striatum relative mass and proteins promoted by methylphenidate. To the best the authors' knowledge, this is the first time that the interference in synaptic plasticity after chronic exposure to environmental levels of methylphenidate is shown.

Key words: Ecotoxicology, emerged pollutants, wastewater, neurotoxicology.

INTRODUCTION

The inappropriate discard of chemical residues, named emerged pollutants, in wastewater raises concern (Geissen et al., 2015). There are significant amounts of residues such as medicines present in the aquatic environment (Ramirez et al., 2009; Stahl et al., 2009). However, these chemicals are not commonly monitored or removed despite their potential to cause adverse effects (Geissen et al., 2015).

Methylphenidate (MTP) is a cyclized amphetamine derivative currently marketed as Ritalin (Mariotti et al., 2013). The use of MTP has been rising to treat (mis)diagnosed attention deficit hyperactivity disorder

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and also for nonmedical recreational use (as drug of abuse due to its amphetamine nature) or as cognitive enhancers (Burgard et al., 2013; Letzel et al., 2010; Mariotti et al., 2013).

There are consistent reports of MTP aquatic contamination (Burgard et al., 2013; Letzel et al., 2010). Moreover, it is well known that pharmacological exposure to MTP during youth causes neurochemical adaptations such as cerebral oxidative stress, changes in the energetic metabolism and neuronal/synaptic changes in animal models (Comim et al., 2014; Fagundes et al., 2007; Fagundes et al., 2010; Martins et al., 2006; Schmitz et al., 2012; Zehle et al., 2007; Bock et al., 2017). However, the question that remains to be answered is whether exposure to environmental levels of MTP residues promote brain significant changes. Thus, the aim of this work was to evaluate chronic neurotoxicity of MTP residual exposition using rats as animal model.

MATERIALS AND METHODS

Chemicals

Tablets of commercial 10 mg Ritalin were purchased from Novartis, Brazil. Albumin bovine standard and malondialdehyde were purchased from Sigma-Aldrich (Brazil). Methanol, triethylamine, and phosphoric acid were purchased from Merck (Brazil). All chemicals used in high performance liquid chromatography (HPLC) analysis were of analytical grade.

Animal model and experimental design

Wistar male rats, aging 21-days old and weighting 39.9±4.2 g were used. Animals were housed in a temperature- and humidity-controlled environment. Food and water were provided *ad libitum* and animals were subjected to a 12 h light/dark cycle. Animal experiments were approved by the Ethical Committee of University of Passo Fundo (Protocol number 10/2014). Housing and experimental treatment of the animals were in accordance with the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Research. All experiments complied with current Brazilian laws.

Animals were followed from day 21 (day 1 of the experiment) to day 60 (day 40 of the experiment), which corresponds to the childhood and adolescence of development stages from rats (Teixeira-Gomes et al., 2015). Rats were divided into the following groups (8 animals per group): Control (receiving tap water ad libitum), MTP 0.1875 µg/L (receiving tap water contaminated with 0.1875 µg/L MTP *ad libitum*) and MTP 1.875 µg/L (receiving tap water contaminated with 1.875 µg/L MTP *ad libitum*). Solutions were weekly prepared through trituration of 1 tablet of 10 mg Ritalin. Powder was solubilized in 100 mL tap water. Further dilutions were performed to reach working concentrations of 0.1875 and 1.875 µg/L of MTP. Working concentrations were defined considering environmental relevant concentrations previously described (Burgard et al., 2013).

During the experiment, daily clinical evaluations of all animals were performed. The parameters evaluated were: Piloerection, dehydration, hemorrhage and diarrhea, motor function (tone and movement coordination) breathing (rate and depth, gasping), mucosal color (pale, cyanotic), and clinical signals of abdominal pain. Individual weight and consumption of food and water were also evaluated until the end of the study.

On day 40 of the study (when animals completed 60-days old and starts the young adulthood) (Teixeira-Gomes et al., 2015), rats were anesthetized with xylazine/ketamine (10 and 100 mg/kg, respectively) and euthanized by exsanguination.

Assessment of MTP stability

MTP stability in water was assessed though HPLC analysis, as previously described with some adaptions (Franck et al., 2009). MTP quantification was performed using Flexar Perkin Elmer liquid chromatograph equipped with autosampler and PDA UV-vis detector. Chromatographic separation was achieved using C18 column Perkin-Elmer (250 × 4.6 mm, 5 µm) and mobile phase consisted of methanol:phosphoric acid 0.025% (80:20) with a final pH adjusted to 5.9 with trimethylamine. Flow rate, injection volume and wavelength were 1.2 ml/min, 20 µL, 206 nm, respectively. Total run time was 10 min.

Neurotoxicity assay

Cerebral striatum, cortex, and hippocampus were washed in a phosphate-buffered saline solution, pH 7.5, dried, and weighted to assess the relative mass of the cerebral region (calculated as a percentage of the total body-weight on the day of euthanasia).

Cerebral regions were homogenized (1:10 m/v) in phosphate-buffered solution, pH 7.5, with Ultra-Turrax®. The protein levels were determined by the Lowry method as previously described (Lowry et al., 1951; Rossato et al., 2014). Lipid peroxidation rate was assessed through the thiobarbituric acid reactive species reaction (Calegari et al., 2015; Ohkawa et al., 1979). Results are expressed as µmol/mg of protein.

Statistical analysis

Results are presented as means ± standard deviation. Normality distribution was assessed through D'Agostino and Pearson test. Statistical comparison were performed with Kruskal-Wallis test (one-way ANOVA on ranks) followed by Dunn's post hoc test. The evaluations of the rat relative body weight gain and the consumptions of food and water were followed daily; thus, the statistical analysis was performed using repeated measures ANOVA followed by the Tukey's post hoc test. Significance was accepted at p values < 0.05 *vs* control.

RESULTS

MTP stability

MTP is stable in water for at least 7 days (when rats received a new bottle of water containing MTP freshly prepared). No additional chromatographic peak was observed, which could be attributed to degradation products (data not shown).

MTP systemic effects

During the experiment, animals from all groups increase body weight until day 25. From day 25 until the end of the experiment, animals exposed to MTP (1.875 µg/L)

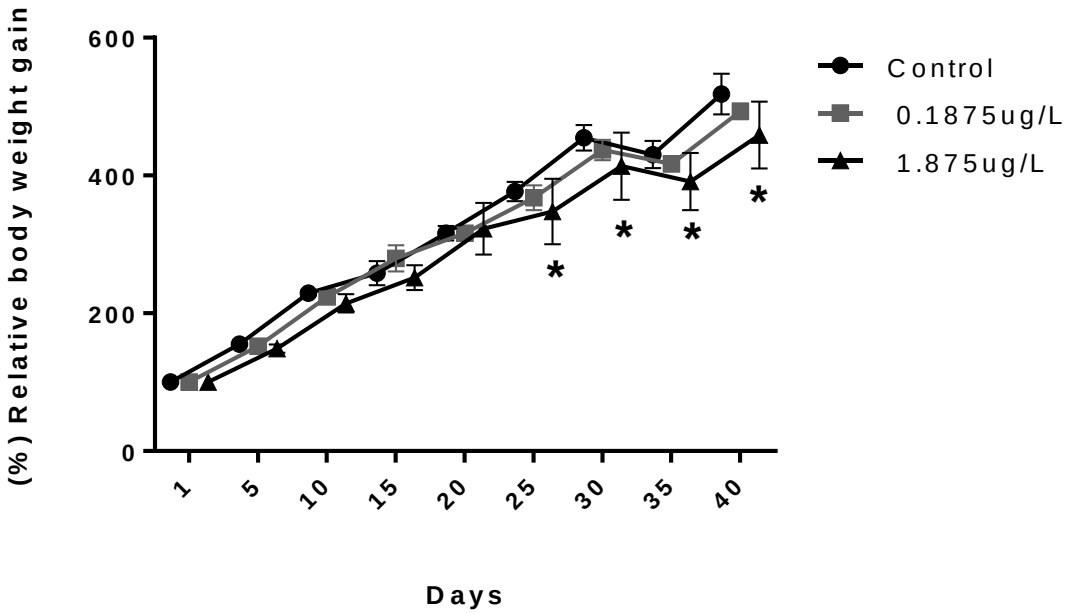

Figure 1. Relative body weight gain (%) of animals chronically exposed to MTP in water during childhood and adolescence. Results are presented as mean ± standard deviation. Statistical analysis was performed using repeated measures ANOVA followed by Tukey's post hoc test (**p*<0.05 *vs* Control).

significantly reduce relative body weight gain compared to control ($p<0.05$) (Figure 1). This change is not accompanied by significant alterations in clinical parameters or in food or water consumptions (data not shown).

MTP exposure impact on brain architecture

Rats exposed to relevant MTP residues (1.875 µg/L) in water during childhood and adolescence present a significant increase in striatum relative weight (Figure 2A) and striatum protein content (Figure 2B), for both concentrations, compared to control ($p<0.05$). A decrease in cortical relative weight is also observed, however, it is not statistically significant ($p>0.05$, Figure 3A). Moreover, this chronic exposition significantly decrease cortical protein content ($p<0.05$, Figure 3B). Hippocampus protein content and relative mass of MTP-exposed rats remain unchanged compared to control group (data not shown). Present exposition to MTP-residues does not cause changes in lipid peroxidation rates in any of the cerebral regions evaluated.

DISCUSSION

The major finding of the present work is the evidence that the chronic exposure to MTP contaminated water, at concentrations similar to those found in the environment, elicits changes in neurodevelopment (Figures 2 and 3). MTP chronic-induced neurotoxicity was already shown previously (Adriani et al., 2006; Daniali et al., 2013; El-Zein et al., 2005; Fagundes et al., 2007; Fagundes et al., 2010). However, to the best of our knowledge, this is the first time that changes of brain structures are demonstrated using low concentrations, consistent with what is found in the environment.

Water containing MTP was changed every week for another contaminated solution freshly prepared. During this period (7 days), MTP is stable, as verified through HPLC analysis. Thus, toxicological effects observed here are attributed to MTP and not to some degradation product. Considering pharmacokinetics parameters, after a pharmacological dose of 10 mg, 1% MTP is eliminated in its unaltered form and 80% as ritalinic acid, its inactive metabolite (Burgard et al., 2013). Wastewater analysis revealed the presence of 1.5 µg/L of ritalinic acid (Burgard et al., 2013). Relevant doses of MTP, employed in the present work, were set based in the logarithmic scale considering human excretion profile.

In this work, the exposure occurred since post-natal day 21 to post-natal day 60 of rats. This period comprises the childhood and the adolescence of rats (Teixeira-Gomes et al., 2015). It is known that this phase of neurodevelopment is characterized by neurobiological process which will influence behavior and skills in the adulthood (Chang et al., 2007; Teixeira-Gomes et al., 2015). Striatum, which contains the highest densities of dopaminergic synapses (Chang et al., 2007), is the most affected cerebral region (Figure 2). It is tempting to speculate that the increase in weight and protein content

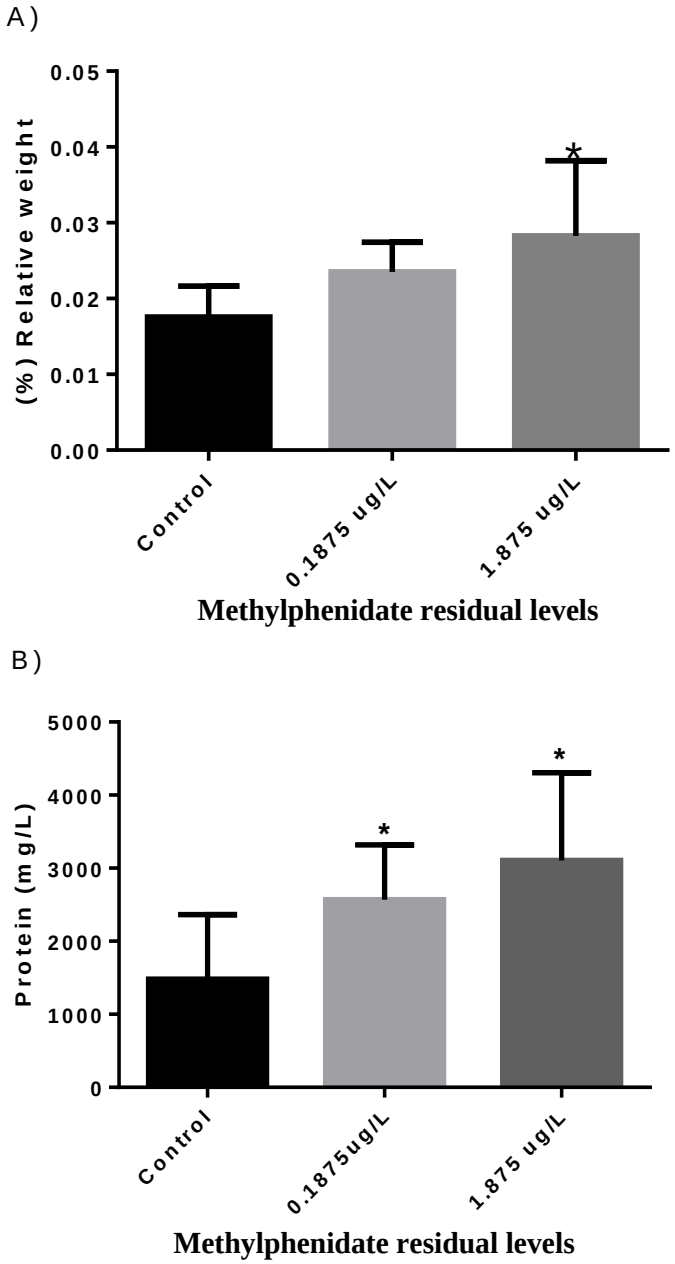

Figure 2. Striatum changes caused by chronic exposure to MTP residues in water. (A) Striatum relative weight and (B) Striatum protein content. Results are presented as mean ± standard deviation. Statistical analysis was performed using Kurskal-Wallis test followed by Dunn's multiple comparisons test (*p<0.05).

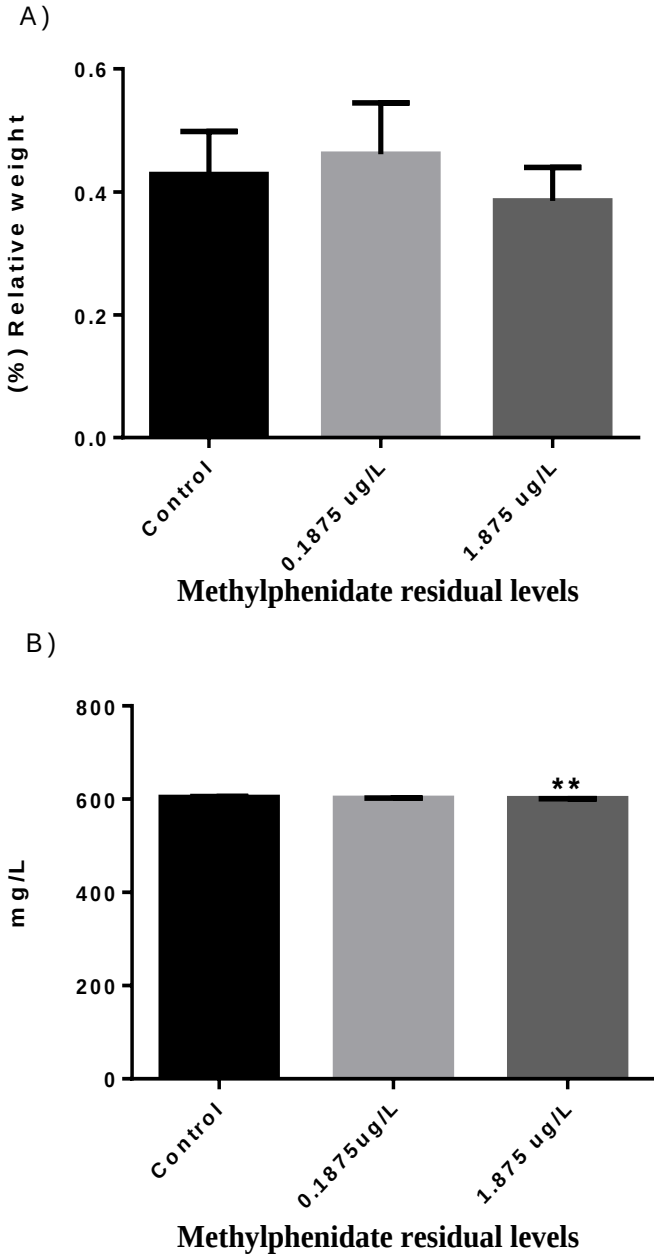

Figure 3. Cortical changes caused by chronic exposure to MTP residues in water. (A) Cortical relative weight and (B) Cortical protein content. Results are presented as mean ± standard deviation. Statistical analysis was performed using Kurskal-Wallis test followed by Dunn's multiple comparisons test (*p<0.05).

observed in the striatum after MTP treatment may be caused by MTP-induced elevations in brain metabolic activity (Bock et al., 2017) and in stimulation of genes encoding for proteins involved in neuronal and synaptic growth. Rats treated during adolescence (30 to 40-days old) with MTP in pharmacological doses (2 mg/kg, daily via intraperitoneal) presented upregulation of more than 700 genes in the striatum. These changes are involved in synaptic plasticity and interfere with normal formation maturation, and stabilization of new neural connections (Adriani et al., 2006). In addition, the chronic elevation of dopamine in the brain may exert trophic effects by itself and/or by other substances released by microglia and astrocyte (Chang et al., 2007) and thereby interferes with neuronal growth.

This is in line with reports demonstrating that MTP

treatment during prepuberty results in an increase of dendritic complexity (“hypertrophy” in the anterior cingulate cortex (Zehle et al., 2007). In the present regimen of exposition, it is shown that striatum enlargement is related to increase protein level (Figure 2). Thus, striatum increased relative weight is unlikely related only to water content due to cellular edema, reinforcing the trophic mechanism.

In cortical tissue, significantly decreased protein levels after MTP chronic exposition was observed (Figure 3). Consistently to cortical changes observed here, juvenile rats treated with MTP at higher doses (5 mg/kg, from postnatal day 7 to postnatal day 35) showed decreased density of norepinephrine transporter in the cortex (Gray et al., 2007). This region is crucial to executive functions and decision making (Gray et al., 2007), thus structural and functional changes may significantly impact adult life.

One of the most common adverse effects of MTP is weight-loss due to anorectic potential (Leddy et al., 2004). Despite the reduction in body weight gain (Figure 1), no significant differences in food intake were observed between groups, suggesting no involvement of appetite suppression.

In the present regimen of exposition, no changes were observed in lipid peroxidation rate in any of the cerebral regions evaluated. However, significant changes in oxidative status after MTP was already described before in studies using higher doses (Fagundes et al., 2007; Fagundes et al., 2010; Martins et al., 2006). Acute administration of higher doses of MTP (5 mg/kg) to 25-days old rats decreased complex I activity in cerebellum and cortex. When chronically administered (1, 2 or 10 mg/kg for 28 days), this change was not observed (Fagundes et al., 2010). It is known that the extension of oxidative damage is also age-dependent, being young brains more susceptible than adult brains (Martins et al., 2006).

It was demonstrated that even relatively low environmentally relevant concentrations of MTP elicit changes in weight and protein content of important cerebral regions, with the most pronounced effects seen in the striatum. Metabolic and cognitive consequences of these changes remains to be elucidated but it might involve motor movements, reinforcement pathway, executive functions and decision making.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adriani W, Leo D, Guarino M, Natoli A, Di Consiglio E, De Angelis G. Traina E, Testai E, PERRONE-CAPANO C.A.R.L.A, Laviola G (2006). Short-term effects of adolescent methylphenidate exposure on brain striatal gene expression and sexual/endocrine parameters in male rats. Ann N. York Acad Sci. 1074(1):52-73.

Bock J, Breuer S, Poeggel G, Braun K (2017). Early life stress induces attention-deficit hyperactivity disorder (ADHD)-like behavioral and brain metabolic dysfunctions: functional imaging of methylphenidate treatment in a novel rodent model. Brain Struct. Funct. 222(2):765-780.

Burgard D, Fuller R, Becker B, Ferrell R, Dinglasan-Panlilio M (2013). Potential trends in attention deficit hyperactivity disorder (ADHD) drug use on a college campus: wastewater analysis of amphetamine and ritalinic acid. Sci. Total Environ. (450-451):242-249.

Calegari E, Endres H, Dallegrave E, Cendron L, Bertol C, Siqueira L, Rossato L (2015). Silymarin elicits partial protection against methotrexate-induced hepatotoxicity in wistar rats. Int. J. Pharm. Pharm Sci. 7(9):462-465.

Chang L, Alicata D, Ernst T, Volkow N (2007). Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. Addiction 102(SUPPL. 1):16-32.

Comim CM, Gomes KM, Réus GZ, Petronilho F, Ferreira GK, Streck EL, Dal-Pizzol F, Quevedo J (2014). Methylphenidate treatment causes oxidative stress and alters energetic metabolism in an animal model of attention-deficit hyperactivity disorder. Acta Neuropsychiatrica, 26(2):96-103.

Daniali S, Nahavandi A, Madjd Z, Shahbazi A, Niknazar S, Shahbazzadeh D (2013). Chronic ritalin administration during adulthood increases serotonin pool in rat medial frontal cortex. Iranian Biomed J, 17(3):134-139.

El-Zein R, Abdel-Rahman SZ, Hay MJ, Lopez MS, Bondy ML, Morris DL, Legator MS. (2005). Cytogenetic effects in children treated with methylphenidate. Cancer Lett. 230(2):284-291.

Fagundes AO, Aguiar MR, Aguiar CS, Scaini G, Sachet MU, Bernhardt NM, Rezin GT, Valvassori SS, Quevedo J, Streck EL (2010). Effect of acute and chronic administration of methylphenidate on mitochondrial respiratory chain in the brain of young rats. Neurochem Res. 35(11):1675-80.

Fagundes AO, Rezin GT, Zanette F, Grandi E, Assis LC, Dal-Pizzol F, Quevedo J, Streck EL (2007). Chronic administration of methylphenidate activates mitochondrial respiratory chain in brain of young rats. Int J Dev Neurosci, 25(1):47-51.

Franck M, Meneghini L, Rossato L, Limberger R, Froehlich P (2009). Development and validation of an LC-UV method for quantitation of 4-bromo-2,5-dimethoxyamphetamine (DOB), 4-bromo-2,5-dimethoxyphenetylamine (2C-B), methylphenidate, fenproporex and amfepramone.Chromatographia 69(S2):143-148.

Geissen V, Mol H, Klumpp, E, Umlauf G, Nadal M, van der Ploeg M, van de Zee SE, Ritsema CJ, (2015). Emerging pollutants in the environment: a challenge for water resource management. Int. Soil Water Conserv. Res. 3(1):57-65.

Gray JD, Punsoni M, Tabori NE, Melton JT, Fanslow V, Ward MJ, Zupan B, Menzer D, Rice J, Drake CT, Romeo RD (2007). Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. J. Neurosci. 27(27):7196-7207.

Leddy JJ, Epstein LH, Jaroni JL, Roemmich JN, Paluch R, Goldfield GS, Lerman C (2004). Influence of methylphenidate on eating in obese men. Obes. Res. 12(2):224-232.

Letzel M, Weiss K, Schüssler W, Sengl M (2010). Occurrence and fate of the human pharmaceutical metabolite ritalinic acid in the aquatic system. Chemosphere 81(11):1416-1422.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurment with the Folin phenol reagent. J. Biol. Chem. 193(1):265-272.

Mariotti KC, Rossato LG, Fröehlich PE, Limberger RP (2013).

Amphetamine-Type Medicines :A Review of Pharmacokinetics, Pharma-codynamics , and Toxicological Aspects. Curr. Clinic Pharm. 8(4):350-357.
Martins M, Reinke A, Petronilho F, Gomes K, Dal-Pizzol F, Quevedo J (2006). Methylphenidate treatment induces oxidative stress in young rat brain. Brain Res. 1078(1):189-197.
Ohkawa H, Ohishi N,Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95(2):351-358.
Ramirez AJ, Brain RA, Usenko S, Mottaleb MA, O'Donnell JG, Stahl LL, Wathen JB, Snyder BD, Pitt JL, Perez-Hurtado P, Dobbins LL (2009). Occurence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. Environ. Toxicol. Chem. 28(12):2587-2597.
Rossato LG, Costa VM, Dallegrave E, Arbo M, Dinis-Oliveira RJ, Santos-Silva A, Duarte JA, Lourdes Bastos M, Palmeira C, Remião F(2014). Cumulative mitoxantrone-induced haematologic and hepatic adverse effects in a sub-chronic in vivo model. Basic Clin. Pharmacol. Toxicol. 114(3):254-262.
Schmitz F, Scherer E, Machado F, Da Cunha A, Tagliari B, Netto C, Wyse A (2012). Methylphenidate induces lipid and protein damage in prefrontal cortex, but not in cerebellum, striatum and hippocampus of juvenile rats. Metabolic Brain Disease 27(4):605-612.
Stahl L, Snyder B, Olsen A, Pitt J (2009). Contaminants in fish tissue from US lakes and reservoirs: A national probabilistic study. Environ. Monit. Assess 150(1-4):3-19.
Teixeira-Gomes A, Costa V, Feio-Azevedo R, Bastos M, Carvalho F, Capela J (2015).The neurotoxicity of amphetamines during the adolescent period. Int. J. Dev. Neurosci. 41:44-62.
Volkow ND, Ding YS, Fowler JS, Wang GJ, Logan J, Gatley JS, Dewey S, Ashby C, Liebermann J, Hitzemann R, Wolf AP (1995). Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. Arch Gen Psychiatr. 52(6):456-463.
Zehle S, Bock J, Jezierski G, Gruss M, Braun K (2007). Methylphenidate treatment recovers stress-induced elevated dendritic spine densities in the rodent dorsal anterior cingulate cortex. Dev. Neurobiol. 67(14):1891-900.

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