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Rotenone and paraquat perturb dopamine metabolism: A computational analysis of pesticide toxicity

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ABSTRACT

Pesticides, such as rotenone and paraquat, are suspected in the pathogenesis of Parkinson's disease (PD), whose hallmark is the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*. Thus, compounds expected to play a role in the pathogenesis of PD will likely impact the function of dopaminergic neurons. To explore the relationship between pesticide exposure and dopaminergic toxicity, we developed a custom-tailored mathematical model of dopamine metabolism and utilized it to infer potential mechanisms underlying the toxicity of rotenone and paraquat, asking how these pesticides perturb specific processes. We performed two types of analyses, which are conceptually different and complement each other. The first analysis, a purely algebraic reverse engineering approach, analytically and deterministically computes the altered profile of enzyme activities that characterize the effects of a pesticide. The second method consists of large-scale Monte Carlo simulations that statistically reveal possible mechanisms of pesticides. The results from the reverse engineering approach show that rotenone and paraquat exposures lead to distinctly different flux perturbations. Rotenone seems to affect all fluxes associated with dopamine compartmentalization, whereas paraquat exposure perturbs fluxes associated with dopamine and its breakdown metabolites. The statistical results of the Monte-Carlo analysis suggest several specific mechanisms. The findings are interesting, because no *a priori* assumptions are made regarding specific pesticide actions, and all parameters characterizing the processes in the dopamine model are treated in an unbiased manner. Our results show how approaches from computational systems biology can help identify mechanisms underlying the toxicity of pesticide exposure.

1. Introduction

Pesticides, such as rotenone and paraquat, have been suggested as contributors to the pathogenesis of Parkinson's disease (PD), the 2nd most common neurodegenerative disorder (Brown et al., 2006; Costello et al., 2009; Giasson and Lee, 2000; Gorell et al., 1998; Le Couteur et al., 1999; Priyadarshi et al., 2000; Tanner and Goldman, 1996; Wang et al., 2011). In animal models, paraquat exposure can cause a loss of dopaminergic neurons and lead to an aggregation of α-synuclein (Brooks et al., 1999; McCormack et al., 2002), and rotenone exposure can reproduce many of the typical features of PD (Betarbet et al., 2000; Sherer et al., 2001, 2003). Although the association between pesticide exposure and PD has been established, the actual, specific impacts of pesticides on dopaminergic neuron function are not clear. Paraquat has a chemical structure similar to the neurotoxin 1-methyl-4-phenylpyridinium ion (MPP+), a reaction product of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is primarily known as an impurity of some illicitly manufactured recreational drugs (Kopin, 1987). Based on this chemical similarity, paraquat toxicity has been attributed to its inhibitory effect on mitochondrial complex I (Cocheme and Murphy, 2008). However, it has been argued that paraquat toxicity is not mediated through the dopamine transporter (DAT), which is required in MPP+ induced loss of dopaminergic neurons (Richardson et al., 2005). Moreover, paraquat is not a substrate for DAT in its native divalent cation state (Rappold et al., 2011), and it is only a very weak inhibitor of mitochondrial complex I with an IC_{50} of 8.1 mM (Richardson et al., 2005). Rotenone can also inhibit mitochondrial

Abbreviations: PD, Parkinson's disease; MPP+, 1-methyl-4-phenylpyridinium ion; DAT, dopamine transporter; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNpc, *substantia nigra pars compacta*; DOPAC, 3,4-dihydroxyphenylacetate; HVA, homovanillic acid; ODEs, ordinary differential equations; DOPAC, 3,4-dihydroxyphenylacetate; Catsup, catecholamines-up.

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complex I (Betarbet et al., 2000; Sherer et al., 2001). However, Betarbet et al. (2000) demonstrated that rotenone exerts uniform inhibition of mitochondrial complex I throughout the brain. In consideration of the distinctive dopaminergic neuronal loss in PD, these observations suggest that mitochondrial complex I inhibition cannot fully explain the preferential toxicity of paraquat and rotenone.

A hallmark of PD is the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc). Thus, for paraquat and rotenone to be causative of PD, they need to specifically target dopaminergic neurons, either directly or indirectly. Mitochondrial complex I inhibition by rotenone and paraquat may induce cell apoptosis, but it lacks the specificity of targeting dopaminergic neurons. Rotenone specifically targets human dopaminergic SH-SY5Y cells, but not breast cancer MCF-7 cells and hepatoma HepG2 cells, although it inhibits mitochondrial complex I in all these cells and produces reactive oxygen species (Greenamyre et al., 2003; Rowlands and Casida, 1998; Watabe and Nakaki, 2007). To establish specific toxicity patterns of pesticide exposure in dopaminergic neurons, recent attention has focused on dopamine metabolism. Watabe and Nakaki used human dopaminergic SH-SY5Y cells to investigate the association between dopamine metabolism and rotenone-induced apoptosis (Watabe and Nakaki, 2007). They proposed that the dopamine redistribution from vesicles to the cytosol may account for rotenone toxicity. Sakka et al. (2003) suggested that dopamine mediates rotenone selective toxicity in the mesencephalon. Rotenone was furthermore suggested to inhibit the enzyme tyrosine hydroxylase (TH), which is the rate limiting enzyme of dopamine synthesis (Hirata and Nagatsu, 2005). The specific toxicity of MPP+ in dopaminergic neurons seems to be associated with the utilization of DAT; however, DAT does not mediate rotenone toxicity, although both toxins (MPP+ and rotenone) inhibit mitochondrial complex I (Hirata et al., 2008). Sai et al. (2008) proposed that rotenone alters dopamine distribution and metabolism, leading to its selective toxicity in dopaminergic neurons. Similarly, Lawal et al. (2010) suggested that rotenone, but not paraquat, targets dopamine storage, with toxic consequences at least in *Drosophila*. The toxic mechanisms of paraquat may be related to oxidative damage through promoting superoxide and hydrogen peroxide, which are normal by-products of dopamine metabolism (Richardson et al., 2005).

To study pesticide action in dopaminergic neurons, we utilize here a computational approach to infer sites within the dopamine pathway system that are potentially targeted by rotenone and paraquat. In contrast to traditional, targeted experimentation, computational systems biology does not necessarily rely on the *a priori* formulation of a specific hypothesis, and often investigates biological questions from a systemic point of view with the help of dynamic models. Over the past years we have developed such models to investigate dopamine homeostasis and dynamics in dopaminergic neurons, as well as dopamine-based signal transduction across synapses (Qi et al., 2008, 2009, 2010a,b, 2011a, 2013). These models can serve as computational platforms for simulations of dopamine synthesis, transport, release, degradation, and reuptake. They can also be utilized to identify "choke points" that are particularly vulnerable to perturbations. In addition, these models have been applied to study dopamine related diseases.

In the present study, we describe how a mathematical model of dopamine metabolism may be used to investigate the specific effects of paraquat and rotenone. While it is clear that pesticides could have multiple aspects of PD-related toxicity, we focus here specifically on perturbations of dopamine metabolism in dopaminergic neurons. Our first approach does not involve an *a priori* hypothesis and is directly based on a top-down analysis of experimental observations characterizing the effects of pesticides on dopamine metabolism. Like with other mathematical models, this approach is employed to obtain unique answers. As a second, complementary approach, we use a Monte Carlo simulation method that reveals potential pesticide action sites in a specific and statistically robust manner. Our findings are predictive and may serve as a basis for guiding and targeting future biological studies of the impacts of pesticides on dopaminergic neurons.

2. Methods

2.1. A mathematical model of dopamine metabolism

Over the past years, we have been developing and refining a series of mathematical models of dopamine metabolism, dopamine-associated signal transduction, and the effects of disease or drug use on normal functioning (Qi et al., 2008, 2009, 2010a,b, 2011a,b, 2012; Voit et al., 2008, 2012; Wu et al., 2011). One of these models serves as the computational platform for the present study; details regarding the dopamine pathway structure and the description of equations have been presented elsewhere (Qi et al., 2008, 2012).

In a nutshell, dopamine is synthesized from the precursor L-DOPA, which is produced from the essential amino acid tyrosine that is made available to the brain through the blood stream. Synthesized dopamine is packed into storage vesicles through the vesicular monoamine transporter VMAT2. Spontaneously, or in response to a stimulus, vesicular dopamine is released into the synaptic cleft where it executes its signaling function. Released dopamine can be carried back to the presynaptic terminal for recycling through the specific transporter DAT. Dopamine can also be enzymatically converted into other metabolites such as 3,4-dihydroxyphenylacetate (DOPAC) and homovanillic acid (HVA). In addition to these fundamental processes, the dopamine model accounts for many secondary processes, as well as regulatory processes, such as inhibition signals that affect certain enzymatic steps.

The model is set up with ordinary differential equations (ODEs) and uses mass action and Michaelis–Menten representations for all biochemical reactions. Most of the numerical values for concentrations, turnover rates, reaction orders, and Michaelis constants were obtained from experimental measurements reported in literature. The few remaining parameters were estimated by fitting the model to experimental data. The parameterized model was tested with standard methods of algebraic and computational diagnostics, as well as a variety of simulation studies, and the reliability and correctness of the model were validated against biological and clinical observations (Qi et al., 2008, 2012).

We performed two types of analyses, which are conceptually different and complement each other. The first analysis, a purely algebraic reverse engineering approach, results in a singular prediction for the action of a pesticide. While elegant, however, it does not offer a measure of reliability and statistical significance. It is also limited to a relatively small number of candidate processes. The second method consists of large-scale Monte Carlo simulations that lead to distributions of possible actions of pesticides, which are assessed statistically. By and large, the two methods yielded consistent results, even though this was not *a priori* guaranteed.

2.2. Pertinent literature information for model analysis

Both the reverse engineering method and the Monte Carlo simulations require information regarding the normal and the pesticide-affected steady states of the metabolites in the dopamine system. The two approaches then infer what alterations of processes have an effect on the system compatible with the perturbed steady-state profiles of the metabolic pathway under pesticide