Structural Stability Analysis of Models of Dopamine Synthesis and D1 Receptor Trafficking in RPT Cells using CRNT

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Dopamine plays an important role in different physiological and metabolic functions, including the control of sodium excretion in the kidney. Studies have shown that there is a positive correlation between a defect in dopamine synthesis and/or dopamine receptor function, and a defect in renal sodium excretion – which may lead to the development of essential hypertension. Specific receptors for dopamine, such as the D1 receptor, have been identified in the various regions within the kidney. It is observed that errors regarding dopamine receptor-G protein coupling and changes in the signaling components may be responsible for the failure of dopamine to increase sodium excretion in hypertensive subjects. In this paper, two symbolic kinetic models of dopamine synthesis and one of dopamine D1 receptor trafficking are presented. The three models are chemical reaction networks constructed and analyzed using Chemical Reaction Network Theory (CRNT), a framework that provides different insights on the static properties of a chemical reaction network regarding the existence of steady states, their multiplicity, and structural stability. It is found that all three networks do not support multiple steady states.

Keywords: chemical reaction network theory, concentration robustness, D1 receptor trafficking, renal dopaminergic system, structural stability analysis, symbolic kinetic modeling

INTRODUCTION

Essential hypertension is a common human disorder characterized by an increase in systemic blood pressure caused by high levels of sodium in the bloodstream. This happens when sodium reabsorption increases in the renal proximal tubule (RPT) cells in the kidney, which in turn occurs when more than the usual amount of sodium is returned to the bloodstream from the glomerular filtrate. Dopamine, an important catecholamine, has been identified to control primary physiological and metabolic processes in the human body – such as locomotion, hormone secretion, behavior

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– as well as various gastrointestinal, renal, and cardiovascular functions. In the kidney, dopamine is seen as an important regulator of blood pressure, sodium balance, and other metabolic functions.

Evidently, there is a positive correlation between a defect in dopamine synthesis and/or dopamine receptor function, and the decrease in sodium excretion that leads to the development of essential hypertension (Zeng et al. 2004). An unusual amount of sodium is reabsorbed by the RPT cells when there is an increase in sodium transport in the RPT, or when there are abnormalities in the dopamine D1 receptors, present in various regions in the kidney. Activation of the D1 receptors ensures that only the proper amount of sodium needed by the body remains in the system. Thus, abnormalities in dopamine reception lead to allowing more than the acceptable amount of sodium to be returned to the bloodstream, paving the way for hypertension.

Due to the function of the RPT cells – and the kidney in general – in the long-term regulation of sodium excretion and blood pressure, many studies have focused on sodium flow in RPT cells in relation to the pathogenesis of essential hypertension. Armando et al. (2011) provide a comprehensive review on the role of dopamine and D1 receptors in blood pressure regulation and renal functions. Furthermore, the mechanisms of dopamine and D1 receptor trafficking in the kidney and its relation to the expression of essential hypertension are also extensively detailed by Zeng et al. (2004). However, there is to date no published mathematical model that describes the dynamics of D1 trafficking in RPT cells, and this is the main motivation of this study.

Furthermore, the authors aim to understand the mechanisms of dopamine synthesis and receptor trafficking in relation to sodium excretion through computational models. One important property that is usually studied in computational models of biochemical interactions is the stability of the system. This refers to a network's ability to maintain its function despite environmental or structural changes. Note that regulating the concentration variations of interacting species within known bounds is an important factor in maintaining function. For such cases, it is necessary to develop methodologies that will identify an appropriate mechanism that is able to reproduce the behavior observed in experiments. One of these frameworks is CRNT, proposed by Fritz Horn, Roy Jackson, and Martin Feinberg in the 1970s (Horn and Jackson 1972; Feinberg 1979, 1980). It is used to describe the behavior of a constructed chemical reaction network and provide analyses regarding the existence, uniqueness, multiplicity, and stability of solutions – independent of the parameters present in the system. The advantage of CRNT over traditional kinetic analysis methods is that it provides a straightforward way to analyze the type of dynamical behavior that one can expect from an arbitrarily complex network of chemical reactions just by the inspection of the structure of the constructed network.

In this paper, we present two networks in the human renal dopaminergic system, the renal dopamine synthesis network, and the dopamine D1 receptor trafficking network; and how CRNT may be applied in their analyses.

**Dopamine Metabolism in RPT Cells**

**Renal dopamine synthesis.** As illustrated in Figure 1, and integrating the processes detailed in Zeng et al. (2004) and Qi et al. (2008), circulating L-DOPA (LDOPA) enters the RPT cell with the help of the transporter LAT2. Internal L-DOPA (LDOPA) is then converted to dopamine by aromatic L-amino acid decarboxylase (AADC). Some of the dopamine (Da) goes to the lumen (DaL), while some exit to the interstitial space (DaI), and bind to D1 receptors located in the respective membranes. The amount of dopamine that remains inside the RPT cell is degraded by catechol-O-methyltransferase (COMT) to 3-methoxytyramine (3-MT), or by monoamine oxidase (MAO) and semicarbazide-sensitive amine oxidase (SSAO) to 3,4-dihydroxyphenylacetaldehyde (DOPAL) – which, in turn, is converted by aldehyde dehydrogenase (ALDH) to 3,4-dihydroxyphenylacetic acid (DOPAC). Homovanillic acid (HVA) is produced by ALDH and MAO from 3-MT. COMT also contributes to the production of HVA by degrading DOPAC. Some of the HVA then leaves the RPT cell, going to the lumen. The metabolite S-adenosyl-L-homocysteine (SALH) inhibits the production of 3-MT and HVA.
Renal Dopaminergic Networks in RPT Cells

**Dopamine synthesis and reception network.** Following the processes discussed in the previous section, the pathway diagram provided in Lubenia (2012) will be used to study the structural stability of the dopamine synthesis network. Two network models are constructed.

The first network, which is a translation from Figure 1, attempts to include all the reactions and metabolites as discussed in Zeng *et al.* (2004) and Lubenia (2012).
\[
\begin{align*}
LDOPA_E + LAT2 & \rightarrow LDOPA_I \\
LDOPA_I + AADC & \rightarrow ALDH : AADC \\
ALDH : AADC + Da & \rightarrow DaL \\
DaL + D_1R_A & \rightarrow DaL : D_1R_A \\
Da & \rightarrow DaI \\
DaI + D_1R_B & \rightarrow DaI : D_1R_B \\
DOPAC + SALH & \rightarrow DOPAC : SALH \\
DOPAC : SALH + COMT & \rightarrow DOPAC : SALH : COMT \\
DOPAC : SALH : COMT + Da & \rightarrow 3MT \\
ALDH + MAO & \rightarrow ALDH : MAO \\
ALDH : MAO + 3MT & \rightarrow HVA \\
MAO + SSAO & \rightarrow MAO : SSAO \\
Da + MAO : SSAO & \rightarrow DOPAL \\
DOPAC & \rightarrow DOPAL + ALDH \\
Da + SSAO & \rightarrow Da : SSAO \\
Da : SSAO + COMT & \rightarrow Da : SSAO : COMT \\
Da : SSAO : COMT + DOPAC & \rightarrow HVA \\
0 & \rightarrow LDOPA_E \\
0 & \rightarrow DOPAC \\
0 & \rightarrow D_1R_A \\
0 & \rightarrow D_1R_B \\
HVA & \rightarrow 0 \\
DaI : D_1R_B & \rightarrow 0
\end{align*}
\]

Legend:

LDOPA_E – external L-DOPA
LAT2 – pH-sensitive type 2 L-type amino acid transporter
LDOPA_I – internal L-DOPA
AADC – aromatic L-amino acid decarboxylase
ALDH – aldehyde dehydrogenase
Da – dopamine
DaL – dopamine in the lumen
D_1R_A – D1 receptor in the lumen
D_1R_B – D1 receptor in the interstitial space
DaI – dopamine in the interstitial space

D_1R_B – D1 receptor in the interstitial space
DOPAC – 3,4-dihydroxyphenylacetic acid
SALH – S-adenosyl-L-homocysteine
COMT – catechol-O-methyltransferase
3MT – 3-methoxytyramine
HVA – homovanillic acid
MAO – monoamine oxidase
SSAO – semicarbazide-sensitive amine oxidase
DOPAL – 3,4-dihydroxyphenylacetaldehyde

For example, Equation 1 corresponds to the process of internalization of external L-DOPA (LDOPA_E) which, with the help of LAT-2 transporter, is then converted to LDOPA_I. The other reaction equations are constructed similarly. For the metabolites that become a complex, say ALDH and AADC, it is written as ALDH:AADC – separated by colons. The reactions of the form ‘0 → X’ mean that metabolite X is produced, while reactions of the form ‘X → 0’ mean that X is degraded.
The second network, which is a simplified version of the preceding model excluding the transporters and other catalytic components (non-boldfaced substances in Figure 1), is constructed to find out the effects of any model reductions in terms of deficiency and other topological characteristics. This is given by the following set of reactions:

\[
\begin{align*}
LDOPA_E & \rightarrow LDOPA_I \\
LDOPA_I + Da & \rightarrow Da \\
Da & \rightarrow Da_L \\
Da & \rightarrow Da_I \\
Da & \rightarrow 3MT \\
Da & \rightarrow DOPAL \\
DOPAL & \rightarrow DOPAC \\
DOPAC & \rightarrow HVA \\
3MT & \rightarrow HVA \\
Da_L + D_1 R_A & \equiv Da_L : D_1 R_A \\
Da_I + D_1 R_B & \equiv Da_I : D_1 R_B \\
0 & \rightarrow LDOPA_E \\
0 & \equiv DOPAC \\
0 & \equiv D_1 R_A \\
0 & \equiv D_1 R_B \\
HVA & \rightarrow 0 \\
Da_L : D_1 R_A & \rightarrow 0 \\
Da_I : D_1 R_B & \rightarrow 0
\end{align*}
\]

Legend:
LDOPAE – external L-DOPA
LDOPAI – internal L-DOPA
Da – dopamine
DaL – dopamine in the lumen
D1R – D1 receptor in the lumen
DaI – dopamine in the interstitial space

\[D_1 R_A – D1 receptor in the interstitial space\]
\[3MT – 3-methoxtyramine\]
\[DOPAL – 3,4-dihydroxyphenylacetaldehyde\]
\[DOPAC – 3,4-dihydroxyphenylacetic acid\]
\[HVA – homovanillic acid\]

**Dopamine D1 receptor trafficking network.** The basic kinetic diagram of D1R trafficking – derived from Zeng et al. (2004) and Armando et al. (2011) – is constructed by combining the canonical receptor availability model in Shankaran et al. (2007a) with the GPCR activation, desensitization, and phosphorylation steps of the model from Woolf and Linderman (2003) and Shankaran et al. (2007b); and the internalization, degradation, and recycling steps of the model from Lauffenburger and Linderman (1996) and Fogler (2005). It is illustrated in Figure 2.

Following the diagram of D1 receptor trafficking presented in Figure 2, a network is constructed for the analysis of the structural stability of the network of interest. This is presented in the following set of reaction equations:
After constructing the chemical reaction networks, where primarily the types of reactions are identified, it sometimes becomes a problem to analyze the qualitative properties of certain networks. This happens when the details of the reaction mechanisms are difficult to describe or are even unknown. However, there might exist experimental evidence such that the system can display a particular dynamic such as stability. For such cases, it is necessary to develop methods that will identify an appropriate mechanism that is able to reproduce the behavior observed in experiments. Graph-theoretic methods are one option. They are able to allow different interpretations of network behavior and are therefore useful in the early stages of the model development where choices between proposed mechanisms need to be made.
CRNT – a mathematical framework initiated by F. Horn and R. Jackson in 1972 and further developed by M. Feinberg and his students (Feinberg 1979, 1980) – is used to describe the behavior of a constructed chemical reaction network and provide analyses regarding the existence, uniqueness/multiplicity, and stability of solutions of the chemical reaction network independent of the parameter values present in the system. The advantage of CRNT is that it provides a straightforward way to analyze the type of dynamical behavior that one can expect from an arbitrarily complex network of chemical reactions just by inspecting the topology of the associated graph. One may check whether a given reaction network has (multiple) equilibrium points without the need for constructing kinetic equations and assigning parameter values to the equations.

The fundamental concept of CRNT is the chemical reaction network, a finite directed graph accompanied by a triple – \{S, C, R\} – that describes how chemical species interact within a system (Feinberg 1979). The triple \{S, C, R\} consists of a set of (chemical) species \(S\), a set of complexes \(C\), and a set of reactions \(R\) – with the notion of species, complexes, and reactions following the law of mass action.

A central concept to CRNT is the discussion on deficiency, as it tells us about the degree of linear independence of the chemical reactions in the network. The deficiency, \(d\), can be computed as:

\[
d = \text{(number of complexes)} - \text{(number of linkage classes)} - \text{(rank of the reaction network)},
\]

where a linkage class is simply defined as a maximal set of connected complexes, and the rank of a network is the number of elements in the largest linearly independent set of reaction vectors for the network. The reader is pointed to Feinberg 1987 for the formal definitions and formulas.

Higher deficiency in networks means that the reactions are less independent from one another and are thus more connected. In other words, the higher the deficiency, the more tightly the reactions in the network are linked to one another. Furthermore, the concept of the deficiency of a network can be applied to linkage classes, as the latter is a subnetwork of the original CRN.

One of the significant results in CRNT is the mass action injectivity property (Craciun and Feinberg 2005). Craciun and Feinberg established that this property is a sufficient, but not necessary, condition for showing the absence of multiple equilibria. They provided a Jacobian-determinant property sufficient for the injectivity of mass action reaction systems on the assumption that each species is subject to a degradation reaction. They also pointed out that if a network possesses the mass action injectivity property, then multiple positive stoichiometrically compatible steady states are impossible, no matter what the rate constants are.

Another advantage of CRNT is that the analysis is implemented in CRNTToolbox (Ji et al. 2011), a Windows program which is freely available and only requires an input set of CRN reactions. Its results provide information, for instance, on whether the associated dynamical system admits multiple steady states for some kinetic parameter values, or that no such combination of parameter values exists.

**Structural Stability Analysis**

**Structural stability analysis of the renal dopamine synthesis network.** The first kinetic diagram, as shown in the previous section, is composed of 36 complexes and 11 linkage classes. The rank of the CRN is 24, which gives a deficiency equal to \(d = 36 - 11 - 24 = 1\). Also, all the 11 linkage classes have deficiency 0.

The second kinetic diagram consists of 17 complexes and one linkage class. The rank of the CRN is 13, which gives a deficiency equal to \(d = 17 - 1 - 13 = 3\).

These values both declare that the dopamine synthesis network is monostable, based on the classification provided in Siegal-Gaskins et al. (2011). This means that a set of rate constants can be found for which the corresponding mass action system possesses a unique steady state, if one exists. A result in Lubenia (2012) confirms that there indeed exists a positive steady state given a set of initial parameters obtained experimentally.

**Structural stability analysis of the renal dopamine D1 receptor trafficking network.** The third kinetic diagram is composed of 28 complexes and nine linkage classes. It is worth noting that it is not a weakly reversible network, which means the
pathways linking participating complexes (including the receptor D₁R) are not strongly connected. Moreover, the rank of the CRN is 15, which gives us a deficiency equal to \( \delta = 28 - 9 - 15 = 4 \). All of the linkage classes have deficiency 0.

From Figure 2, it can be seen that the chemical reaction network in the previous section can be divided into six subnetworks, with each subnetwork pertaining to the six important subprocesses in D₁ receptor trafficking. Reaction 44 refers to D₁ receptor activation, Reactions 45–51 to the interaction with the G proteins, Reactions 52 and 53 to receptor desensitization and phosphorylation, Reaction 54 to internalization, Reaction 55 to ligand-receptor complex recycling, and Reaction 56 to complex degradation. The remaining equations describe the production and degradation of the participating chemical species.

**DISCUSSION**

CRNT has provided a way to work beyond numerical models, for which parameters are sometimes difficult to obtain. At the very least, such as in the case of this study, it assists in exploring how reaction systems are characterized according to some behavior or function.

The two dopaminergic systems described earlier are analyzed in this section. Such analysis will provide insights on how the models will behave under different initial conditions – as well as on relationships among topological parameters – which in this case are the number of linkage classes and the number of terminal linkage classes, as related to their network dynamics.

**Dopamine Synthesis**

Note that the transporters and other catalytic components induce the reactions, but do not participate in them, thus the construction of a second network. In the CRNT point of view, the corresponding analysis is highly dependent on the network topology; thus, it is better to consider two models and check their respective network topologies.
The CRNToolbox gives the following analysis for both networks: “…the corresponding differential equations cannot admit a steady state at which all species concentrations are positive, nor can they admit a cyclic composition trajectory that passes through a point at which all species concentrations are positive.”

This shows that the reduction of the model in this case does not significantly affect network dynamics. However, note that the difference between the number of terminal linkage classes \( t \) and the linkage classes \( l \) (i.e., \( t - l \)), increased. The first network has \( t - l = 12 - 11 = 1 \), while the second network \( t - l = 3 - 1 = 2 \). Though \( t > l \) in both cases, we have either \( t - l = \delta \) (first network) or \( t - l < \delta \) (second network). Based on results from Feinberg (1987), the fact that \( t - l \leq \delta \) makes it still possible for the kinetic subspace – the smallest linear space containing the image of the species formation rate function – to equal the stoichiometric subspace. Most results in CRNT so far are for the case \( t - l \leq 0 \), which implies that the kinetic and stoichiometric subspaces are equal; if \( t - l > \delta \), then the kinetic and stoichiometric subspaces are not equal.

In the range of both models, since there are rate constants for which the kinetic subspace is equal to the stoichiometric subspace, some kind of structural stability holds. In other words, if \( t - l = \delta \) and the system has a positive steady state, then the kinetic subspace is smaller than the stoichiometric subspace, with all the consequences. On the other hand, there is a degenerate behavior that can occur if the kinetic subspace is smaller than the stoichiometric subspace in a mass action system. When the kinetic subspace for a mass action system is smaller than the stoichiometric subspace, a stoichiometric compatibility class containing at least one positive steady state will usually contain an infinite number of positive steady states (Feinberg 1987).

**D1 Receptor Trafficking**

The kinetic equations, as enumerated in the previous section, provide insights on the different species and reactions that influence the availability and internalization of dopamine and/or D1 receptors. However, due to the limited availability of literature regarding the quantitative relationships of the various interacting chemical species and subprocesses, subsequent parametric analysis (i.e., stability and sensitivity analyses) is suggested for further research so as to extend the preliminary analyses provided by Villar (2012).

Furthermore, the structural stability of the constructed reaction network is examined using Higher Deficiency Theory (Ellison 1998, Ji 2011) and implemented in CRNToolbox (Ji et al. 2011). The conclusion is that both networks cannot admit multiple (positive) steady states or a degenerate steady state. This is also supported by the Mass-Action Injectivity Test, proposed by Craciun and Feinberg (2005, 2006), and implemented in CRNToolbox, which concludes that the second network is injective i.e., there is no set of rate constants for which the resulting reaction system can admit two distinct stoichiometrically-compatible steady states or a degenerate steady state.

The D1R trafficking network is monostable based on the classification provided by Siegal-Gaskins et al. (2011), which assures that there exists a set of rate constants for which the corresponding mass action system possesses a unique positive steady state, if any. However, it is difficult to present any possible set of reaction rates that will result in a monostable network due to the unavailability of parameters that can be used to solve the associated kinetic equations that will illustrate the trafficking dynamics.

Another finding is that the number of linkage classes and the number of terminal linkage classes are equal i.e., \( t - l = 0 \). This implies that the kinetic and stoichiometric subspaces of the D1 receptor trafficking network are equal. In this case, no matter what rate constants are assigned to the reactions, the corresponding mass action differential equations admit at most one steady state in each positive stoichiometric compatibility class. This result is the basis of the “classical” or deficiency-oriented theory of CRNT, where it is only applicable to a class of reaction systems under mass action kinetics.

**CONCLUSION**

This paper applies CRNT as a tool for analyzing two renal dopaminergic systems in human proximal tubule cells: renal dopamine synthesis and dopamine D1 receptor trafficking. Both renal dopaminergic systems yield monostable networks, which mean that there is a possibility of a unique steady state given kinetic parameters. On one point, these findings are significant contributions to molecular systems biology research as they may be the first analyses of the
said networks. However, they are made more significant by the fact that they were arrived at despite the limitation that most kinetic parameters, such as reaction rates and kinetic orders, are neither easily available from literature nor easy even to estimate.

Both renal dopaminergic systems yield monostable networks, which means that there is a possibility of a unique steady state given kinetic parameters. This implies that, theoretically, there is at most only one set of parameters \( i.e., \) biochemical measures, that the system will converge to and achieve as its steady state. If a steady state is arrived at through some computational means, the CRNT finding of monostability assures that it will be the only such state for the network.

Since CRNT is unable to give the exact parameter values for a steady state, one must rely on the verification of findings with actual experiments and the corresponding interpretation with respect to sodium excretion.

Thus, the future directions of this research are two-fold: verification of the findings, as detailed above, and further development of the theoretical framework of CRNT. The first case is important to the experimenters as this would enhance their confidence in using the framework in formulating preliminary analyses about the system, and in identifying crucial species and reactions in the network. The latter pertains to a call for more concrete and comprehensive propositions and analyses about the network given a particular structure. CRNT is relatively young as a mathematical framework and is in itself a rich area of research to work on. Clearly, this opens up a myriad of possibilities in aiding the solution of molecular systems biology problems that both mathematicians and biologists may individually work on and, ultimately, collaborate on.

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