Multi-biomarker pharmacokinetic-pharmacodynamic relationships of central nervous systems active dopaminergic drugs

Brink, W.J. van den

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

Diseases of the Central Nervous System (CNS) decrease the quality of life of millions of people worldwide (1–3). A lot of time, effort and resources are therefore put into the development of CNS drugs, while the success rates are low. For example, in the period from 2003 – 2011, almost 400 CNS drugs entered phase I clinical development, while less than 10% of them received market approval (4). The main reasons for these low success rates are the lack of understanding of the complexity of the brain, the presence of the blood-brain-barrier limiting drug penetration, CNS mediated side effects, and the lack of good biomarkers that represent the interaction of the drug with neurophysiological systems (5–7).

Systems pharmacology aims to integrate multiple biological systems for the evaluation pharmacological effects to improve the understanding and the prediction of drug effects (8–12). While several examples show the merits of systems pharmacology (13,14), they are driven by a priori insights into detailed pharmacological knowledge. This is not always available during early drug development. As an alternative, the pharmacometabolomics approach in combination with multivariate statistical methods provides an unbiased and data-driven way to evaluate the system-wide drug effects at the level of biochemical pathways (15–17). However, in order to understand and extrapolate the typically non-linear drug effects, one needs to quantify the relation between drug dose and response using pharmacokinetic/pharmacodynamic (PK/PD) modeling (18–20). In other words, it will be important to divert from a fully empirical approach towards a mechanistic approach, without loosing the unbiased and data-driven properties of pharmacometabolomics. Additionally, given the limited access to the brain in terms of sampling, it is important to discover blood-based biomarkers that represent drug effects in the brain. In this thesis, we therefore asked two questions:

1. How can we quantify the relation between drug dose and the dynamic systems response *in vivo*?
2. How can we obtain blood-based markers that represent central drug effect?

Section I – General introduction

As an initial step, in **Chapter 2**, these questions were placed in the context of translational pharmacology of CNS drugs with particular focus on interspecies scaling. On one hand, metabolomics enables comprehensive evaluation of interspecies differences at the biochemical level (21–23). On the other hand, mechanistic PK/PD modeling in combination with allometric and physiology-based scaling is used to extrapolate drug responses from one species to another (18,19). Moreover, mechanistic PK/PD modeling can potentially be applied to describe the relation between neurological drug effects and blood-based biomarkers, as will be discussed below. PK/PD-metabolomics modeling was proposed as an integration of PK/PD and pharmacometabolomics with the potential to increase understanding and

extrapolative ability during translational drug development (Figure 1). In **Chapter 3**, a systematic search was performed in PubMed to investigate the pathways involved in dopaminergic drug effects, as well as the availability of blood-based biomarkers related to these pathways. A multitude of pathways appeared to be associated with dopaminergic drug effects. This included the neurotransmitter, the nitric oxide and the kynurenine pathway in the brain, as well as neuroendocrine and energy pathway responses in the periphery. Although this may partially be attributed to the lack of selectivity of dopaminergic drugs (24,25), also selective drugs appeared to perturb multiple pathways (26–28). Additionally, we found no studies describing the relation between drug effects in the brain and blood-based biomarkers, except for prolactin. Moreover, pharmacological effects were typically evaluated in a static manner, with no quantification of the dynamics underlying the dose response relation. On basis of these two chapters, we identified three goals an integrative PK/PD-metabolomics method needs to fulfill in order to answer our questions:

- Longitudinal measurement of a systems biomarker response with multiple dose levels included
- Simultaneous evaluation of drug concentrations and drug response biomarkers in plasma and the brain
- Integration of PK/PD principles into pharmacometabolomics data analysis

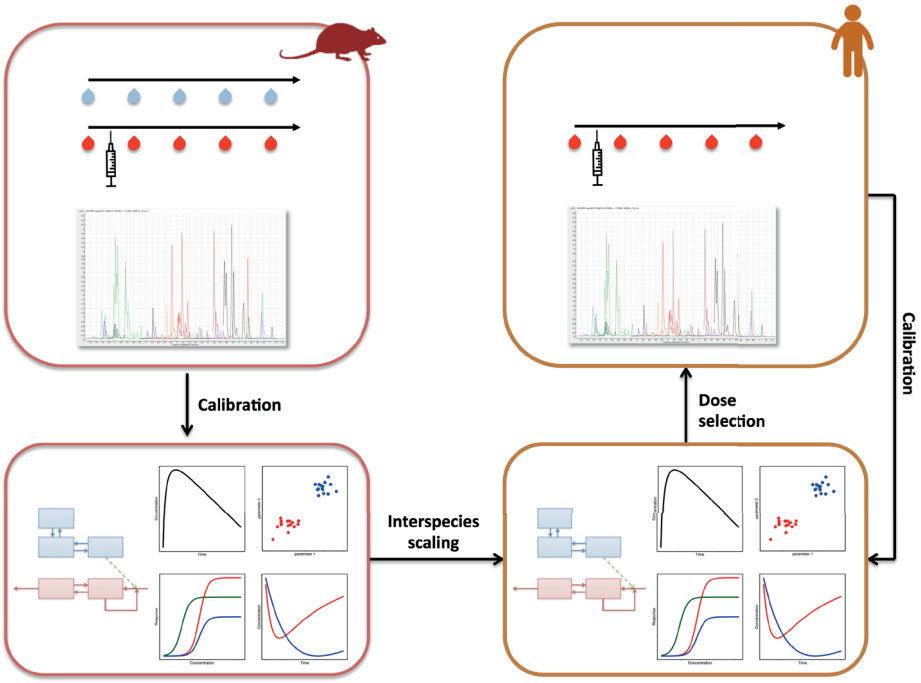

Figure 1. Proposed workflow of PK/PD metabolomics in interspecies scaling Modified from (56) with permission of Taylor and Francis.

Section II – The dynamical neuroendocrine systems response to study dopamine D_2 drug effects

One way to discover blood-based biomarkers is to evaluate the neuroendocrine systems response to CNS drug administration (Figure 2). The neuroendocrine system is a connection between the neural system and the endocrine system, composed of the hypothalamus, the pituitary and the distant endocrine organs. Neural projections from the hypothalamus to the pituitary, for example the tuberoinfundibular dopamine (TIDA) neurons, are controlled by neurochemicals, such as dopamine, serotonin or acetylcholine. These neurons then release signal (e.g. dopamine) into the pituitary to regulate the release of hormones (e.g. prolactin) from the anterior pituitary into the circulation (29). Additional to this mechanism, hypothalamic neurons can also release neuropeptides (e.g. oxytocin) directly into the circulation from their end-feet located in the posterior pituitary. As one of the blood-based biomarker strategies depicted in Figure 2, this principle has been used to evaluate dopaminergic drug effects with prolactin as blood-based biomarker (30–33).

Although widely applied with proven applicability in biomarker-driven drug development, a single-biomarker approach has limitations. As we have seen, dopaminergic drugs exhibit multiple effects on the neuroendocrine system. To anticipate a broader *in vivo* pharmacological profile, we set out for a multi-biomarker approach that can reflect the dynamic endocrine systems response to dopamine drug administration. In **Chapter 4** we investigated the feasibility of a dynamical neuroendocrine systems response upon administration of the dopamine D_2 antagonist remoxipride. Interestingly, only adenocorticotropic hormone (ACTH) and prolactin showed a response, while brain-derived neurotropic factor (BDNF), follicle-stimulating hormone (FSH), growth hormone (GH), luteinizing hormone (LH), thyroid stimulating hormone (TSH) and oxytocin remained unaffected by remoxipride (Table I). The number of neuroendocrine biomarkers responding to the dopamine D_2 agonist quinpirole was also low as we showed in **Chapter 5**, although now also GH and TSH responded in addition to ACTH and PRL (Table I). Considering that the dopaminergic system is biologically connected to more pituitary hormones and neuropeptides than those identified in our studies, for example FSH and LH (34), it is likely that the underlying biological networks are resilient to dopaminergic perturbation by remoxipride and quinpirole. Also, the absence of remoxipride effect on GH and TSH release suggests no basal D_2 receptor activation by endogenous dopamine. Considering the higher affinity of quinpirole to the D_2 receptor relative to endogenous dopamine, it is indicated that only high levels of D_2 receptor activation influence the release. Further studies with multiple dopamine agonists and antagonists are required to validate this series of neuroendocrine markers that can evaluate pharmacological perturbation of the dopaminergic system.

Table I. Overview of neuroendocrine responses after remoxipride and quinpirole administration

	Remoxipride	Quinpirole
Alpha MSH	NA	0
Beta Endorphin	NA	0
Neurotensin	NA	0
Orexin A	NA	0
Oxytocin	0	0
Substance P	NA	0
ACTH	+	+
BDNF	0	0
FSH	0	0
GH	0	-
LH	0	0
PRL	+	-
TSH	0	-

NA: not measured

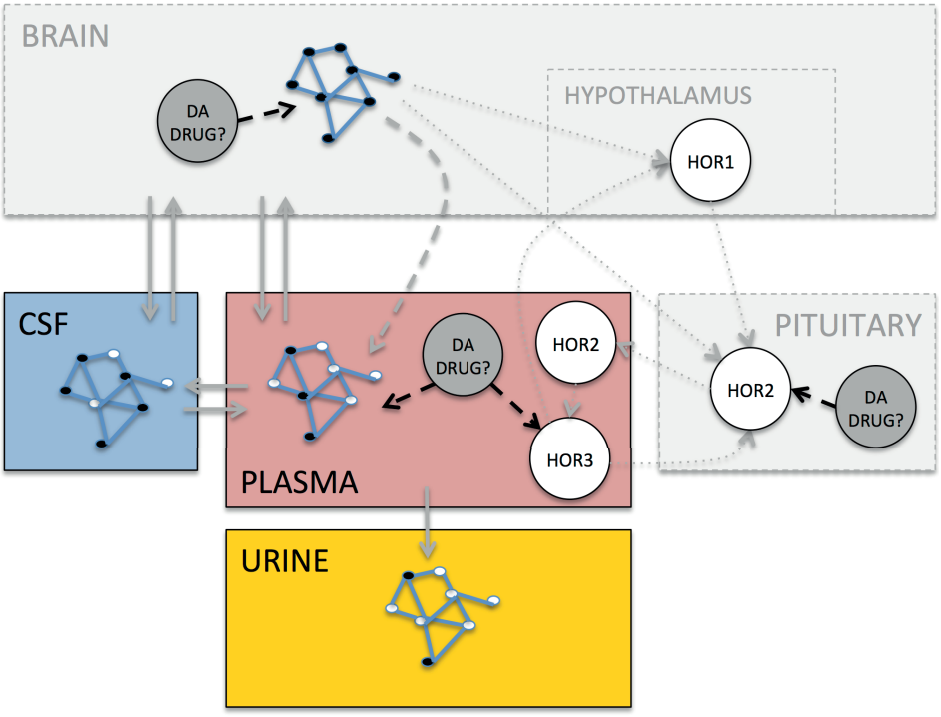

Figure 2. Different physiological mechanisms through which blood-based biomarkers may be related to pharmacological effects in the brain. Modified from (57) with permission of Springer.

An important question is how the hormone-specific potencies are to be interpreted. Two factors influence the potency: receptor affinity and signal transduction efficiency (35). For remoxipride, prolactin was assumed to be the 'gold standard' as biomarker for D_2 receptor activation. While ACTH was found controlled by dopamine in a D_2 specific manner, remoxipride would inhibit its release following this mechanism (36). Interestingly, an increase of ACTH release was observed in **Chapter 4**. Therefore, ACTH likely represents an off-target effect. Concretely, it was concluded that the effect was possibly mediated via the adrenergic receptor, given the ratio of potencies $EC_{50,ACTH}/EC_{50,PRL}$ being similar to the ratio of receptor affinities $k_{i,\alpha 2}/k_{i,D2}$ (37) (**Chapter 4**). While this is an example of the affinity driving the differences in potency estimates, **Chapter 5** shows how signal transduction efficiency determined the differences in potency estimates with quinpirole. Indeed, we could well describe the ACTH, GH, prolactin and TSH responses assuming equal affinity (k_A), but different maximal effect (E_M) and signal transduction efficiency (τ) (Equation 1, Figure 3, **Chapter 5**). Interestingly, τ could be assumed being related to D_2 receptor expression on the hormone releasing 'troph' cells in the pituitary (Equation 2), showing that the signal transduction efficiency can be driven by the characteristics of a specific biological subsystem.

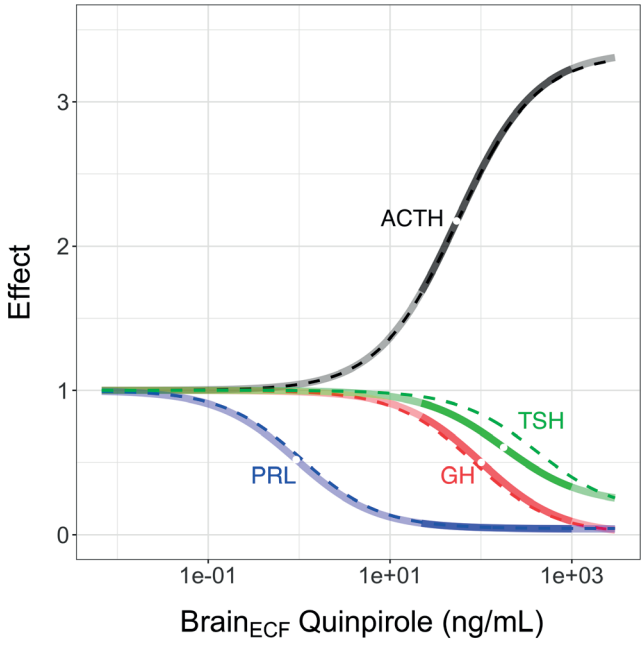

Figure 3. Simulated concentration-effect relation profiles between quinpirole and ACTH, PRL, GH and TSH (solid lines) fitted by equation 1 and 2 (dashed lines)

$$E = \frac{E_m * \tau * C}{k_A + (1 + \tau) * C} \quad (1)$$

$$\tau = \tau_0 * e^{slp * receptor\ expression} \quad (2)$$

Additional to single-administration biomarker responses, **Chapter 5** also presented the effects of longer-term quinpirole administration. This is potentially important for drugs with intended chronic use, amongst which antiparkinson D_2 agonists. Indeed, the effect of D_2 agonists may be subject to sensitization or tolerance as was shown in rats (38). The basal levels of ACTH and prolactin were changed after 8-day administration relative to a single administration. Moreover, not only the basal levels were changed, but also the potency of quinpirole affecting the TSH release. Thus, homeostatic feedback mechanisms may cause changes in basal biomarker levels, as well as the responsiveness of biomarkers to the drug. Interestingly, the biogenic amine and amino acid responses were not changed with longer-term quinpirole administration (**Chapter 7**). This suggests that the systems related to biogenic amines and amino acids are more resilient than the neuroendocrine system to longer-term quinpirole treatment.

As a final remark on using the neuroendocrine system to identify blood-based biomarkers of pharmacological action in the brain, we would like to discuss the topic of target site of drug action. The pituitary is not protected by the blood-brain-barrier, and hence, it is exposed to plasma drug concentrations. While we could statistically identify brain extracellular fluid ($brain_{ECF}$) as target site for the effect of remoxipride on prolactin, also confirmed by others (31), this was not possible for ACTH. Neither could we draw such conclusion for the effect of quinpirole on ACTH, GH, prolactin and TSH. In case of quinpirole, we assumed that with 5 times higher concentrations around the D_2 receptor in the brain as in plasma (i.e. $Kp,uu = 5$), the brain influence would be dominant. However, we acknowledge that the hormone release likely is influenced both at the level of the hypothalamus and the pituitary. The current lack of simultaneous drug and dopamine concentration data in both tissues hampers the development of a model describing a two-level influence of the drug. However, the collection of this type of data appears possible; others have performed microdialysis sampling in the hypothalamus (39) and the pituitary (40), enabling the quantitation of dopamine and drug levels in both tissues.

Altogether, these chapters included longitudinal measurement of a neuroendocrine systems biomarker, after multiple doses of a D_2 antagonist and a D_2 agonist. By applying a PK/PD evaluation to these data, we gained quantitative insights into the neuroendocrine response to dopaminergic drugs and relate those to drug-specific and system-specific pharmacological characteristics.

Section III – The dynamical biochemical systems response to study dopamine D_2 drug effects

In section III the multi-biomarker approach was extended from a neuroendocrine platform with up to 15 hormones and neuropeptides to a metabolomics platform containing 76

amino acids and biogenic amines (41). Several statistical methodologies had been developed dealing with time-resolved high-dimensional (e.g. metabolomics) data. Clustering, for example, is a useful method to identify the main longitudinal patterns in a multivariate dataset (42,43). However, in our experiments we intended not only to include the dimension of time, but also the dimension of dose and sampling site (i.e. plasma and $brain_{ECF}$), in a parallel study design. A multivariate method that can include multiple dimensions, such as time, dose and sampling site, ANOVA-simultaneous component analysis (ASCA) was developed (44). It has been applied to study the effects of dose and time on a metabolomics response in osteoporosis arthritis guinea pigs receiving different dose levels of vitamin C. While this method filled the gap of taking into account underlying study design factors in multivariate statistics, it did not apply very well to our data. In contrast to the guinea pig data, our sampling times are very close and unevenly spaced. Therefore, the successive data points are correlated in a non-linear manner. Treating these time points as factor would limit the identification of the underlying dynamics. More importantly, none of the existing methods dealing with multivariate dynamical patterns integrates pharmacological principles into the data analysis. Therefore, we set out to integrate PK/PD principles into multivariate data analysis. In **Chapter 6**, we measured time-resolved biogenic amine and amino acid patterns upon administration of several remoxipride dose levels. Then, using a three-step approach of i) fitting a turnover model to each single biomarker; ii) clustering the metabolites on basis of the pharmacological parameter estimates; and iii) fitting a turnover model to each cluster of biomarkers, we identified 6 different PK/PD patterns in the data. The *in vivo* potency values related to the clusters were estimated to be 0.0027, 0.019 or 0.12 µM, indicating multiple pathways involved in remoxipride pharmacology. Although we cannot indicate whether these differences were related to off-target effects or differences in signal transduction efficiency, either way this PK/PD-metabolomics model provided a way to define a therapeutic range on basis of a systems response. Furthermore, from the 44 analytes that could be robustly analyzed, 18 were identified as potential biomarker for further validation. While this was a step forward shifting the fully empirical multivariate statistical methods towards a mechanistic modeling approach, the PK/PD-metabolomics method lacked one important feature. No information was included on the biomarker responses in the brain. Unfortunately, at the time, the measurement of biogenic amine and amino acid response in $brain_{ECF}$ by means of intracerebral microdialysis appeared not robust enough. In **Chapter 7**, however, after optimization of the microdialysis method for biogenic amine and amino acid analysis, simultaneous biomarker measurements in $brain_{ECF}$ and plasma were included. Using model comparison metrics, the target site of action related to the individual biomarkers was identified. The clustering step, now both on the $brain_{ECF}$ and the plasma response, was different from that applied in **Chapter 6**. While the remoxipride responses could all be described by turnover models, a larger variety of models, including pool models, turnover models and transit compartment mod-

els, was needed for the quinpirole responses. Therefore, parameter-based clustering not being possible, the clustering was based on simulated biomarker patterns. This clustering approach was proven successful by a good fit of the cluster patterns, as well as the single metabolite patterns by the cluster-based PK/PD model. From this chapter, there are three important conclusions to draw. First of all, even considering that the plasma quinpirole concentrations are 5 times lower than those in $brain_{ECF}$, there are multiple effects observed with plasma as target site. Second, while these biomarker responses originate in other tissues than the brain, most of them are propagated to the brain via transport over the BBB by various transporters. Thus even a drug that does not penetrate the brain, might cause secondary responses in the brain (Figure 4). Third, although multiple biomarker responses were observed in $brain_{ECF}$, only a few of them were transported over the BBB into plasma as a potential blood-based biomarker (Figure 4).

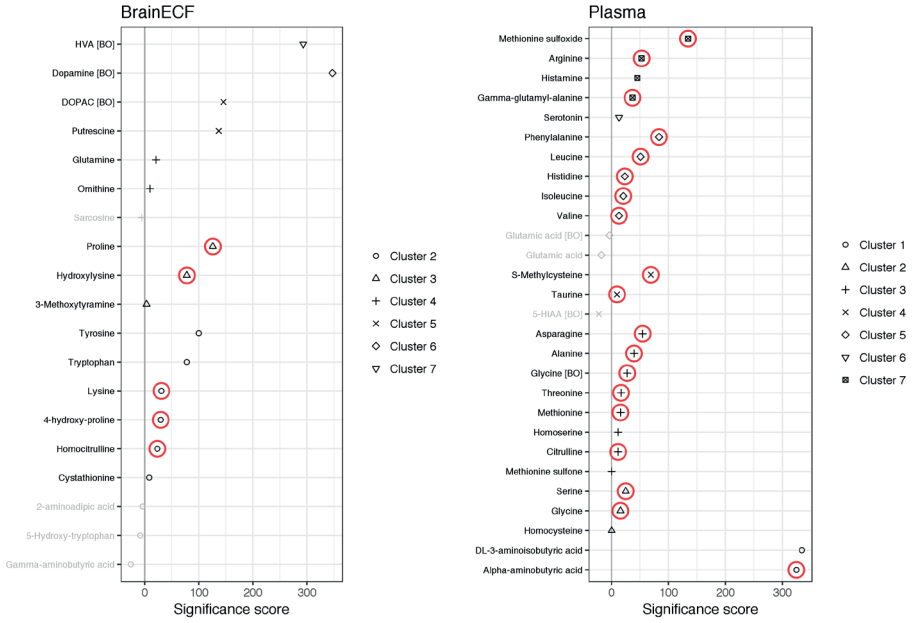

Figure 4. Potential biomarkers of quinpirole effect in $brain_{ECF}$ (left) or plasma (right), positioned right from the vertical grey line. Red circles indicate the biomarkers that distribute over the blood-brain-barrier.

Counter-intuitively, many of the amino acids and biogenic amines that decreased upon remoxipride treatment, were also inhibited by quinpirole. Since in both studies a control group receiving saline was included, the responses must be attributed to drug action. A possible explanation could be that the responses of either remoxipride or quinpirole are caused via another target than the D_2 receptor. Quinpirole has high affinity for the D_2 and the D_3 receptor. There is, however, no reason to believe that D3 receptor opposes D2

receptor, such that D3 receptor agonism leads to similar actions as D2 receptor antagonism. Alternatively, remoxipride possibly interacts with the adrenergic receptor at high concentrations (**Chapter 3**); however, in **Chapter 6** the highest dose was reduced by 50%. Interestingly, remoxipride has higher affinity as an antagonist for the sigma receptor than for the D_2 receptor (37,45,46). Sigma receptor ligands have been investigated in clinical trials for several indications, including diarrhea (agonists) and schizophrenia (antagonists) (47,48). Activation of the sigma receptor reduced the motility of the ileum (49). Antagonism of the sigma receptor, possibly leading to increased motility of the ileum, may lead to shorter intestinal transit time and the concordant reduction of amino acid uptake. Furthermore, the sigma receptor inhibits NMDA receptor sensitization by phosphorylation of the NR1 subunit (50). As an antagonist, remoxipride may thus have disinhibited NMDA receptor sensitization, with the reduction of the NMDA co-activators glycine and serine as a consequence of negative feedback. The reduction of biogenic amine and amino acid levels caused by remoxipride may thus be mediated via the sigma receptor. Although a definitive answer to this matter remains elusive, it underlines the need for extension of our work with other dopamine ligands to identify the responses that are specifically related to D_2 receptor interaction.

Altogether, in **Chapter 6** and **Chapter 7**, we have developed a methodology that accounts for the pharmacological principles underlying the relation between the drug dose and the systems biomarker response. Concretely, we could identify unique *in vivo* concentration-effect relations, target site of drug action, and potential blood-based biomarkers representing the systems response.

We have shown how the PK/PD-metabolomics method, in combination with serial blood and $brain_{ECF}$ sampling and multiple dose levels included, enables the identification of multiple concentration-effect relations and the concordant target site of drug action, and potential blood-based biomarkers that represent these pharmacological properties. Furthermore, as a step towards further mechanistic insight, in a targeted analysis on the neuroendocrine system, we could reveal the relation between drug response, signal transduction efficiency and D_2 receptor expression on the pituitary hormone secreting cells. This positions the PK/PD-metabolomics method in between the unbiased, yet empirical multivariate statistical methods and the mechanism-based quantitative systems pharmacology (QSP) approaches (Figure 5).

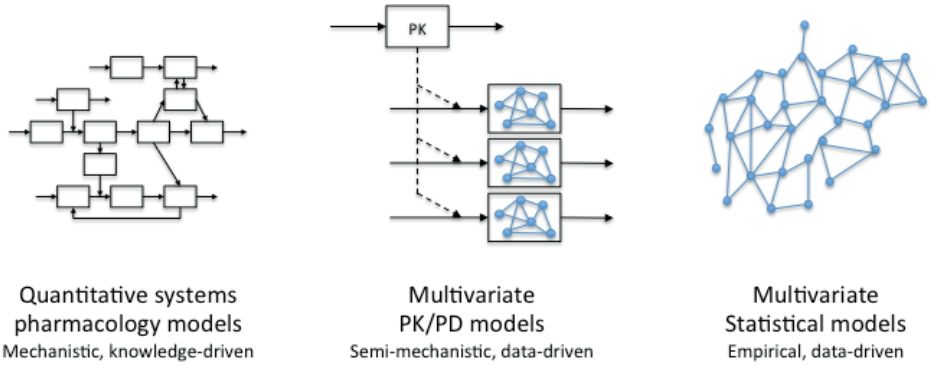

Figure 5. Positioning of PK/PD-metabolomics models between quantitative systems pharmacology (QSP) models and multivariate statistical models

Perspectives of the PK/PD-metabolomics method in CNS drug development

CNS drug development is hampered by poor understanding of pharmacological mechanisms underlying the drug effects on one hand, and lack of (blood-based) biomarkers representing these mechanisms on the other hand. Given that insight into pharmacological mechanisms are strongly associated with the success in clinical drug development (20), there is a need for methodologies that enable early investigation of these mechanisms. The PK/PD-metabolomics method has the potential to increase early insights into pharmacological mechanisms in an unbiased and integrated manner. Metabolomics analysis can easily be added to the standard battery of analysis performed in (pre-)clinical studies. Currently, the metabolomics analysis of one sample costs between the 25 and 400 euros, depending on the sensitivity and the number of analytes. A hypothetical study with 50 subjects and 20 samples per subject will thus cost between 25,000 and 400,000 euros. In the context of the costs of late attrition, this is negligible (4).

PK/PD-metabolomics could have an advantage from early drug discovery to late drug development. During early drug discovery, drugs are typically selected on basis of their affinity to the target of interest. However, it is argued that the efficacy of many CNS drugs is related to multi-target affinity, rather than the selectivity for a single-target (24). Multivariate analysis of *in vitro* receptor affinity profiles of anti-Parkinson drugs revealed subclusters of the D_2 agonists, and it was suggested that other receptors were also involved in the efficacy of these drugs. PK/PD-metabolomics could provide the basis of the correlation between these *in vitro* receptor affinity profiles and the *in vivo* potency profiles to further investigate the relation between the *in vitro* binding fingerprint and the *in vivo* systems effects.

During early drug development, PK/PD-metabolomics will be of value for the discovery of biomarkers, as well as their characterization in terms of pharmacological parameters. Moreover, we have shown how blood-based biomarkers can be discovered with metabolomics analysis performed both in $brain_{ECF}$ and plasma. These biomarkers will be of great value for CNS drug development, given the limitations of human brain sampling. Ultimately, PK/PD-metabolomics could provide the basis of interspecies scaling of a systems-biomarker response as tool in the guidance of the first-in-human dosing regimen. The subsequent validation of the PK/PD-metabolomics model on human metabolomics data could create insights into interspecies differences relevant for drug development.

Finally, in this thesis inter-individual variation was mostly assumed not present given the standardized experimental design. However, inter-individual variation is a key element in precision medicine during clinical development (51). PK/PD-metabolomics is easily extended to describe personal drug responses in order to optimize dosing regimen in an individualized manner. For example, the reduction of TSH by D_2 agonists is known to exacerbate the clinical condition of hypothyroidism patients (52). Knowing the quantitative relation between D_2 agonist dose and the TSH response will enable personalized dosing guidance preventing an unacceptable reduction of TSH levels.

Further development of the PK/PD-metabolomics method

While we have shown the potential of the PK/PD-metabolomics method, we would like to make a few recommendations for further research.

Application of the PK/PD-metabolomics method to other D_2 ligands and clinical validation for proof-of-concept

This thesis has shown the development and feasibility of the PK/PD-metabolomics approach, which now is ready for further validation to generate a proof-of-concept. As we discussed earlier, the comparative results of the D_2 agonist quinpirole and the D_2 antagonist remoxipride appeared non-intuitive, possibly because non-dopaminergic responses are involved. Therefore, to ensure the discovery of dopamine system specific biomarkers, we recommend applying the PK/PD-metabolomics method to multiple D_2 ligands to reveal the overlapping systems biomarker responses. Furthermore, as we argued in **Chapter 2** the PK/PD-metabolomics method has potential to bridge the lack of mechanistic biomarkers that can be used across preclinical and clinical drug development. In **Chapter 7** we showed how a combination of microdialysis and PK/PD-metabolomics enables the identification of blood-based biomarkers. As visualized in Figure 1, an important future step will be the clinical validation of these biomarkers.

Microdialysis in multiple brain locations to study regional responses in the brain
In our experiments, microdialysate samples were collected from the caudate putamen to evaluate biomarker responses representing the function of striatal neurons. A higher level of complexity is presented in the form of brain circuitry that would allow for a regional evaluation of CNS pharmacology (7,14). An interesting next step will be to connect those circuits to the underlying biochemical processes. We recommend the simultaneous evaluation of biochemical responses in multiple brain regions, such as the caudate putamen, the prefrontal cortex or the nucleus accumbens, in order to evaluate the biochemistry related to the circuit functionality. Simultaneous microdialysis sampling in multiple brain regions has proven feasible for CNS PK studies (53).

Additionally, cerebrospinal fluid (CSF) sampling should be included in future PK/PD-metabolomics studies. CSF-based biomarkers have the advantage over blood-based biomarkers that they are not blocked by the BBB. Indeed, in our study with quinpirole, we found many $brain_{ECF}$ biomarkers not reflected in plasma. CSF might provide a good alternative.

Application of PK/PD-metabolomics to measurements from multiple analytical platforms
The PK/PD-metabolomics methodology was developed on basis of the biogenic amine and amino acid metabolomics platform. The choice of this platform was based on our expectation that many of these biomarkers would respond to dopamine ligands. Indeed, we identified many of them responding to remoxipride and quinpirole. However, many other biomarkers, for example, lipids and acylcarnitines, may also be included in the future to study the effects of CNS drugs on multiple biochemical pathways. Our analyses were limited to biogenic amines and amino acids because of limited microdialysate sample volume. Indeed, with microdialysis, there is a compromise between sample volume, time resolution and recovery of the biomarker into the microdialysate. Fortunately, developments at the microdialysis-metabolomics interface are continuously increasing, also focusing on improving the sensitivity of analytical methodology (54,55).

Inclusion of sex differences in the study design
While in our studies only male animals were included for purposes of standardization, there is clear evidence for the impact of sex on biological pathway functionality in disease and drug effect (56,57). Indeed, sex difference was one of the factors that limited the comparison of studies in **Chapter 3** and **Chapter 5**. As an example, the interaction between dopamine or dopamine agonists and the pituitary D_2 receptor is influenced by estrogen, that is expressed in much higher levels in females than in males (58,59). Given the importance of sex differences in disease and drug effect, it will be important to include sex as a variable in clinical and preclinical studies.

General conclusion

We set out to quantify the relation between drug dose and the dynamic systems biomarker response, as well as to discover blood-based biomarkers that represent drug effects in the brain. To that end, we developed the PK/PD-metabolomics method for identification of the main PK/PD patterns in the data. These PK/PD patterns were described in terms of pharmacologically relevant parameters, such as E_{MAX} and EC_{50}, enabling inter- and extrapolation of the systems biomarker response. For the neuroendocrine system, with more knowledge available on the physiological processes involved, we could obtain further mechanistic insights, relating signal transduction efficiency to D_2 receptor expression in the pituitary. Furthermore, with time-resolved metabolomics data available in both $brain_{ECF}$ and plasma, PK/PD-metabolomics enabled the identification of the target site of drug effect for the different biomarkers, as well as the discovery of blood-based biomarkers of drug effects in the brain. Being positioned between the general multivariate statistical methods and QSP models, PK/PD-metabolomics will be useful to provide quantitative pharmacological insights into the systems response of CNS drugs in a data-driven manner.

References

1. Feigin VL, Abajobir AA, Abate KH, Abd-Allah F, Abdulle AM, Abera SF, et al. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017;16(11):877–97.
2. Whiteford HA, Ferrari AJ, Degenhardt L, Feigin V, Vos T. The global burden of mental, neurological and substance use disorders: An analysis from the global burden of disease study 2010. PLoS One. 2015;10(2):1–14.
3. Vigo D, Thornicroft G, Atun R. Estimating the true global burden of mental illness. The Lancet Psychiatry. Elsevier Ltd; 2016;3(2):171–8.
4. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotech. 2014;32(1):40–51.
5. Pangalos MN, Schechter LE, Hurko O. Drug development for CNS disorders: strategies for balancing risk and reducing attrition. Nat Rev Drug Discov. 2007;6(7):521–32.
6. Hurko O, Ryan JL. Translational research in central nervous system drug discovery. NeuroRx. 2005;2(October):671–82.
7. Soares HD. The use of mechanistic biomarkers for evaluating investigational CNS compounds in early drug development. Curr Opin Investig Drugs. 2010;11(7):795–801.
8. van der Graaf PH. CPT: Pharmacometrics and Systems Pharmacology. CPT Pharmacometrics Syst Pharmacol. 2012;1:e8.
9. Geerts H, Spiros A, Roberts P, Carr R. Quantitative systems pharmacology as an extension of PK/PD modeling in CNS research and development. J Pharmacokinet Pharmacodyn. 2013;40(3):257–65.
10. Dumas ME, Davidovic L. Metabolic Profiling and Phenotyping of Central Nervous System Diseases: Metabolites Bring Insights into Brain Dysfunctions. J Neuroimmune Pharmacol. 2015;10(3):402–24.
11. Greef J Van Der, Mcburney RN. Rescuing drug discovery: in vivo systems pathology and systems pharmacology. Nat Rev Drug Discov. 2005;4:961–8.
12. Danhof M. Systems pharmacology - Towards the modeling of network interactions. Eur J Pharm Sci. The Author; 2016;94:4–14.
13. Geerts H, Spiros A, Roberts P, Carr R. Has the time come for predictive computer modeling in CNS drug discovery and development? CPT pharmacometrics Syst Pharmacol. 2012;1(November):e16.
14. Qi Z, Yu GP, Tretter F, Pogarell O, Grace AA, Voit EO. A heuristic model for working memory deficit in schizophrenia . Biochim Biophys Acta. Elsevier B.V.; 2016;1860:2696–705.
15. Burt T, Nandal S. Pharmacometabolomics in Early-Phase Clinical Development. Clin Transl Sci. 2016;9(3):128–38.
16. Kaddurah-Daouk R, Weinshilboum RM. Pharmacometabolomics: implications for clinical pharmacology and systems pharmacology. Clin Pharmacol Ther [Internet]. 2014;95(2):154–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24193171
17. Kantae V, Krekels EHJ, Esdonk MJ Van, Lindenburg P, Harms AC, Knibbe CAJ, et al. Integration of pharmacometabolomics with pharmacokinetics and pharmacodynamics: towards personalized drug therapy. Metabolomics. Springer US; 2017;13(1):9.
18. de Lange ECM, Hammarlund-Udenaes M. Translational aspects of blood-brain barrier transport and central nervous system effects of drugs: from discovery to patients. Clin Pharmacol Ther. 2015;97(4):380–94.
19. Danhof M, de Lange ECM, Della Pasqua OE, Ploeger B a., Voskuyl R a. Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling in translational drug research. Trends Pharmacol Sci. 2008;29(4):186–91.
20. Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. Drug Discov Today. Elsevier Ltd; 2012;17(9/10):419–24.
21. van der Greef J, Adourian A, Muntendam P, McBurney RN. Lost in translation? Role of metabolomics in solving translational problems in drug discovery and development. Drug Discov Today Technol. 2006;3(2):205–11.
22. Blais EM, Rawls KD, Dougherty B V., Li ZI, Kolling GL, Ye P, et al. Reconciled rat and human metabolic networks for comparative toxicogenomics and biomarker predictions. Nat Commun. Nature Publishing Group; 2017;8:1–15.

23. Han J, Dzierlenga AL, Lu Z, Billheimer DD, Torabzadehkhorasani E, Lake AD, et al. Metabolomic Profiling Distinction of Human Nonalcoholic Fatty Liver Disease Progression from a Common Rat Model. Obes (Silver Spring). 2017;25(6):1069–76.
24. Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nat Rev Drug Discov. 2004;3(4):353–9.
25. Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin J-A, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther. 2002;303(2):791–804.
26. Biggs CS, Fowler LJ, Whitton PS, Starr MS. Extracellular levels of glutamate and aspartate in the entopeduncular nucleus of the rat determined by microdialysis: Regulation by striatal dopamine D2 receptors via the indirect striatal output pathway? Brain Res. 1997;753(1):163–75.
27. DeBoer P, Abercrombie ED. Physiological release of striatal acetylcholine in vivo: modulation by D1 and D2 dopamine receptor subtypes. J Pharmacol Exp Ther. 1996;277(2):775–83.
28. Escobar AP, Cornejo FA, Olivares-Costa M, Gonzalez M, Fuentealba JA, Gysling K, et al. Reduced dopamine and glutamate neurotransmission in the nucleus accumbens of quinpirole-sensitized rats hints at inhibitory D2 autoreceptor function. J Neurochem. 2015;134:1081–90.
29. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin : Structure , Function , and Regulation of Secretion. Physiol Rev. 2000;80(4):1523–631.
30. Petty RG. Prolactin and antipsychotic medications: Mechanism of action. Schizophr Res. 1999;35:67–73.
31. Stevens J, Ploeger BA, Hammarlund-Udenaes M, Osswald G, Graaf PH van der, Danhof M, et al. Mechanism-based PK-PD model for the prolactin biological system response following an acute dopamine inhibition challenge: Quantitative extrapolation to humans. J Pharmacokinet Pharmacodyn. 2012;39:463–77.
32. Movin-Osswald G, Hammarlund-Udenaes M. Prolactin release after remoxipride by an integrated pharmacokinetic-pharmacodynamic model with intra- and interindividual aspects. J Pharmacol Exp Ther. 1995;274:921–7.
33. Friberg LE, Vermeulen a M, Petersson KJF, Karlsson MO. An agonist-antagonist interaction model for prolactin release following risperidone and paliperidone treatment. Clin Pharmacol Ther. 2009;85(4):409–17.
34. Pivonello R, Ferone D, Lombardi G, Colao A, Lamberts SWJ, Hofland LJ. Novel insights in dopamine receptor physiology. Eur J Endocrinol. 2007;156:S13–21.
35. Black JW, Leff P. Operational Models of Pharmacological Agonism. Proc R Soc B Biol Sci. 1983;220(1219):141–62.
36. Borowsky B, Kuhn CM. D1 and D2 dopamine receptors stimulate hypothalamo-pituitary-adrenal activity in rats. Neuropharmacology. 1992;31(7):671–8.
37. Köhler C, Hall H, Magnusson O, Lewander T, Gustafsson K. Biochemical pharmacology of the atypical neuroleptic remoxipride. Acta Psychiatr Scand Suppl. 1990;358:27–36.
38. Tirelli E, Jodogne C. Behavioral sensitization and tolerance to the D2 agonist RU 24213: Dissociation between several behavior patterns in mice. Pharmacol Biochem Behav. 1993;44(3):627–32.
39. Li H, Yan Z. Analysis of amino acid neurotransmitters in hypothalamus of rats during cerebral ischemia–reperfusion by microdialysis and capillary electrophoresis. Biomed Chromatogr. 2010;24:1185–92.
40. Granveau-Renouf S, Valente D, Durocher A, Grognet J-M, Ezan E. Microdialysis study of bromocriptine and its metabolites in rat pituitary and striatum. Eur J Drug Metab Pharmacokinet. 2000;25(2):79–84.
41. Noga MJ, Dane A, Shi S, Attali A, van Aken H, Suidgeest E, et al. Metabolomics of cerebrospinal fluid reveals changes in the central nervous system metabolism in a rat model of multiple sclerosis. Metabolomics. 2012;8(2):253–63.
42. Déjean S, Martin PGP, Baccini A, Besse P. Clustering Time-Series Gene Expression Data Using Smoothing Spline Derivatives. EURASIP J Bioinforma Syst Biol. 2007;2007:1–10.
43. Almon RR, DuBois DC, Pearson KE, Stephan DA, Jusko WJ. Gene arrays and temporal patterns of drug response: corticosteroid effects on rat liver. Funct Inegr Genomics. 2003;3(4):171–9.
44. Smilde AK, Jansen JJ, Hoefsloot HCJ, Lamers RJ a N, van der Greef J, Timmerman ME. ANOVA-simultaneous component analysis (ASCA): A new tool for analyzing designed metabolomics data. Bioinformatics. 2005;21(13):3043–8.

45. Gudelsky GA, Nash JF. Neuroendocrinological and neurochemical effects of sigma ligands. Neuropharmacology. 1992;31(2):157–62.
46. Lang A, Soosaar A, Kbks S, Volke K, Bourin M, Bradwejn J, et al. Pharmacological Comparison of Antipsychotic Drugs and sigma-Antagonists in Rodents. 1994;222–7.
47. Voz HP, Stoll KD. Clinical trials with sigma ligands. Pharmacopsychiatry. 2004;37(SUPPL. 3).
48. Teruo H, Shang-Yi T, Tomohisa M, Michiko F, Tsung-Ping S. Targeting ligand-operated chaperone sigma-1 receptors in the treatment of neuropsychiatric disorders. Expert Opin Ther targets. 2011;15(5):557–77.
49. Campbell BG, Scherz MW, Keana JF, Weber E. Sigma receptors regulate contractions of the guinea pig ileum longitudinal muscle/myenteric plexus preparation elicited by both electrical stimulation and exogenous serotonin. J Neurosci. 1989;9(October):3380–91.
50. Yoon SY, Roh DH, Seo HS, Kang SY, Moon JY, Song S, et al. An increase in spinal dehydroepiandrosterone sulfate (DHEAS) enhances NMDA-induced pain via phosphorylation of the NR1 subunit in mice: Involvement of the sigma-1 receptor. Neuropharmacology. Elsevier Ltd; 2010;59(6):460–7.
51. Kohler I, Hankemeier T, Graaf PH Van Der, Knibbe CAJ, Hasselt JGC Van. Integrating clinical metabolomics-based biomarker discovery and clinical pharmacology to enable precision medicine. Eur J Pharm Sci. Elsevier; 2017;109S:S15–21.
52. Haugen BR. Drugs that suppress TSH or cause central hypothyroidism. Best Pract Res Clin Endocrinol Metab. 2009;23(6):793–800.
53. Westerhout J, Ploeger B, Smeets J, Danhof M, Lange ECM de. Physiologically Based Pharmacokinetic Modeling to Investigate Regional Brain Distribution Kinetics in Rats. AAPS J. 2012;14(3):543–53.
54. Zhou Y, Wong J-M, Mabrouk OS, Kennedy RT. Reducing Multifunctional Adsorption to Improve Recovery and In Vivo Detection of Neuropeptides by Microdialysis with LC-MS. Anal Chem. 2015;87(19):9802–9.
55. Zestos AG, Kennedy RT. Microdialysis Coupled with LC-MS/MS for In Vivo Neurochemical Monitoring. AAPS J. The AAPS Journal; 2017;19(5):1284–93.
56. van den Brink WJ, Hankemeier T, van der Graaf PH, de Lange ECM. Bundling arrows: improving translational CNS drug development by integrated PK/PD-metabolomics. Expert Opin Drug Discov. Taylor & Francis; 2018;0(0):1–12.
57. van den Brink WJ, Palic S, Köhler I, de Lange ECM. Access to the CNS: Biomarker Strategies for Dopaminergic Treatments. Pharm Res. 2018;35(3).