Chapter 6
Summary and general discussion
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Introduction

The general aim of this thesis was to improve anti-epileptic drug therapy in brain tumor patients. At the start of this thesis, we gave a broad overview (chapter 1.2) that outlined the challenges that are met in treating tumor-related epilepsy and epileptogenesis. Generally speaking, the first step in the treatment of tumor-related epilepsy is treatment of the tumor itself through surgical resection, irradiation and chemotherapy. However, in most cases of tumor related epilepsy, the effect of tumor treatment on seizure control will not be awaited and (additional) treatment with an antiepileptic drug will be regarded as necessary. It then is pivotal to select an effective drug without major side effects and drug-drug interactions. Frequently, several attempts with various antiepileptic drugs are needed to find the most effective drug with no or few side effects. Unfortunately, till now it is impossible to make an educated choice for the most effective drug for an individual patient based on clinical or molecular markers. As tumor and peritumoral tissue of primary brain tumor patients with epilepsy usually is available (in contrast to almost all patients with non-tumor related epilepsy), primary brain tumor patients may serve as a model to investigate if molecular markers predict response to antiepileptic drugs. Epileptogenesis in brain tumor patients presumably comprises structural, cellular and molecular changes induced by the tumor that lead to changes in the surrounding tissue. Most likely these changes eventually accumulate in alterations in functional connectivity at further distance. Better understanding of focal changes that are involved in epileptogenesis may provide new tools for optimal treatment of the seizures. This knowledge may lead to new targets for antiepileptic drugs or markers that can predict the efficacy of antiepileptic drugs.

In this thesis, we therefore investigated the role of several molecular markers that may be associated with epileptogenesis in primary brain tumor patients, and what their relation is with changes in the neural network of those patients. Eventually, we determined the role of one of those molecular markers as a predictor of response to antiepileptic drug treatment in primary brain tumor patients. What insights have been gained through this thesis?

What makes lesions epileptogenic?

Epileptogenesis concerns a multifactorial pathogenesis and outshines in complexity. In the first section of this thesis, the complexity of epileptogenesis was approached from a lesional point of view. Two pathways that could be related to epilepsy were investigated. The first explored was the synaptic vesicle protein 2A (SV2A) pathway. Although the function of SV2A is not completely understood, it has been suggested that it functions as a calcium regulator in neurotransmitter release and modulates synaptic networks by priming vesicles in quiescent neurons. Loss of SV2A leads to a reduction in action potential-dependent gamma-aminobutyric acid (GABA)ergic neurotransmission. Previous experimental studies in mice have shown that knockout of the SV2A gene leads to abnormal neurotransmission and the development of severe seizures. Furthermore, SV2A has been identified as the binding site for the antiepileptic drug levetiracetam.

In chapter 2.1 the expression of SV2A was investigated in patients with focal cortical dysplasia (FCD) and tuberous sclerosis cortical tubers (TSC), and in human control cortex.
In the dysplastic cortex of both FCD and TSC, neuropil expression for SV2A appeared to be decreased. This study is the first to describe the expression pattern and subcellular localization of SV2A in human cortex. The reduction of SV2A expression within the dysplastic cortex of these highly epileptogenic lesions (as compared to normally organized cortical areas) may potentially contribute to the instability of neuronal networks. Another characteristic feature of SV2A-expression in FCD and TSC cortical tubers was the presence of strong perikaryal expression around dysmorphic neurons. Dysmorphic neurons are hyperexcitable and contribute to epileptic discharges. The presence of high SV2A expression surrounding dysmorphic neurons may reflect disturbance of normal synaptic connection or a compensatory mechanism in areas of increased hyperexcitability.

In the following chapter (chapter 2.2), another pathway that could lead to epilepsy was studied. Recently, it has been suggested that dysfunction of adenosine-mediated neuromodulation plays a role in the development of epilepsy. Under physiologic conditions adenosine acts as an endogenous modulator of synaptic transmission and neuronal activity, mainly by inhibiting neuronal activity. Thus adenosine has anticonvulsant and neuroprotective properties. Adenosine kinase (ADK), expressed by astrocytes, is the key player in the regulation of extracellular adenosine levels in the brain. A role for astrocytic ADK regulation in epileptogenesis has been reported before in mouse models. We observed up-regulation of ADK in activated astrocytes of the hippocampus and temporal cortex of rats after the electrical induction of status epilepticus. In line with these findings, we observed increased expression of astrocytic ADK in the hippocampus and temporal cortex of patients with temporal lobe epilepsy. Two isoforms of ADK have been identified with different subcellular localizations (ADK long and short isoforms with respectively nuclear and cytoplasmic localization). Nuclear ADK is probably involved in epigenetic mechanisms, whereas the cytoplasmic isoform is thought to regulate the extracellular levels of adenosine. Corroborating, mice that over-express the cytoplasmic form of ADK display spontaneous seizures. In our study, ADK expression showed both nuclear and cytoplasmic labeling but with a more prominent cytoplasmic localization. Moreover, we showed that inflammatory molecules such as lipopolysaccharide (LPS) and IL-1β may induce increased expression of ADK in human cultured cells, but the cause of the up-regulation of ADK and the role of inflammation in epileptogenesis remain to be elucidated. Our results indicate that over-expression of ADK is a pathological feature of epilepsy.

Thus, changes in SV2A and ADK expression appear to attribute to epilepsy in lesions. Obviously, a large number of other hypotheses regarding pathophysiology have been put forward. Nevertheless, SV2A and ADK seem particularly interesting in tumor-related epilepsy. SV2A is interesting, since the anticonvulsant action of levetiracetam seems to be mediated by SV2A and levetiracetam is an antiepileptic drug with favorable characteristics for brain tumor patients. Understanding the role of SV2A could lead to better treatment selection in individual patients. Perhaps, SV2A could serve as a predictor of efficacy of levetiracetam. ADK is interesting in tumor-related epilepsy since lots of data also suggest an antiproliferative role of adenosine, implying that over-expression of ADK by tumor cells could be a strategy of those cells to keep growing. Understanding the role and function of ADK in tumor-related epilepsy could provide a new target for antiepileptic drug therapy. Adenosine augmenting therapies might even have the dual benefit of combining antiproliferative with anticonvulsant activity.

Heaving learned this, we were able to extrapolate this knowledge to brain tumors. The question remains: what makes brain tumors epileptogenic?
What makes brain tumors epileptogenic?

In the previous chapters, we investigated two pathways that are involved in epileptogenesis in structural non-tumorous lesions. Both pathways are interesting in the development of brain tumor related epilepsy, since tumors and non-tumorous lesions share a number of similar pathological features suggesting that a common set of underlying mechanisms of epileptogenesis could exist. In the following chapters, the same pathways, as investigated in non-tumorous lesions, were studied in brain tumor patients.

In chapter 3.1, SV2A expression in tumor and peri-tumoral tissue of brain tumor patients with epilepsy was compared to SV2A expression in the tissue of brain tumor patients without epilepsy. We expected a reduction of SV2A expression in the tissue of brain tumor patients with epilepsy in conformity with what we found in non-tumorous lesions as described in chapter 2.1. Surprisingly, the results showed no differences in expression for SV2A between tumor patients with and tumor patients without epilepsy. In glial tumors the peritumoral area has been shown to be the most relevant for the generation and propagation of seizure activity. Since no reduction in SV2A expression was found in peritumoral tissue, the role of SV2A in epileptogenesis in patients with glial tumors remains questionable. However, it is a possibility that not the reduction of SV2A expression, but the loss of function alters neurotransmission and eventually contributes to epileptogenesis in patients with brain tumors.

In Chapter 3.2, we investigated ADK protein expression and function in astrocytic tumors and in peritumoral cortex. Changes in ADK expression and ADK activity were found in tumor astrocytes. Similar to FCD and TSC (chapter 2.2), immunocytochemical analysis showed both nuclear and cytoplasmic labeling, but cytoplasmic expression was predominant. It appeared that ADK expression in the peritumoral cortex of glioma patients with epilepsy was significantly higher than in glioma patients without epilepsy. A key question is whether the increased ADK protein expression leads to an increase in enzymatic activity. Higher levels of ADK activity could be detected in astrocytoma WHO grade III (tumor and peritumoral cortex) compared to control tissue. It is interesting that a previous study found that the concentration of adenosine in the extracellular fluid of tumor and peritumoral tissue was significantly reduced compared to control tissue, suggesting an altered purine metabolism in the tumor area. It has been shown that extracellular adenosine reduces the viability of cultured astrocytoma cells, suggesting that over-expression of ADK might be a strategy of tumor cells to improve survival capabilities. Overall, our results support the role of this enzyme in tumor-associated epilepsy.

Thus, brain tumors and non-tumorous lesions share molecular and cellular changes that might set off epilepsy, suggesting that a common set of underlying mechanisms of epileptogenesis exists. Possibly, deregulation of neurotransmission due to molecular and cellular changes, results in alterations in the functional brain network. But what exactly happens to the brain network and is this a pathophysiological feature of epilepsy?

How do molecular changes due to brain tumors lead to alterations in the whole brain?

This section focused on the role of brain networks in epilepsy and the question if molecular and cellular changes can lead to alterations in the whole brain, subsequently leading to epilepsy. Brain
function can be assessed by using the synchronization of neurons between time series from different brain areas (measured by EEG, MEG or functional MRI): namely functional connectivity. Synchronization of neurons may reflect dynamics related to epilepsy.

In **Chapter 4.1** we investigated whether functional connectivity can be used as a predictor of epilepsy in non-tumor patients. We found differences in EEG functional connectivity between patients with epilepsy and patients without epilepsy after a first seizure. The synchronization likelihood (SL) was used as an index of functional connectivity. Patients with epilepsy showed an increased SL in the theta band when compared to patients without epilepsy. Increased SL in the theta band also proved to be a predictor of the diagnosis epilepsy. The results that we found suggest that interictal brain connectivity in patients with epilepsy differs from the connectivity of patients without epilepsy. The increased synchronization in the theta band may reflect a compensatory mechanism but it also may reflect synchronization disinhibition as a consequence of brain disease.

Brain tumors may also influence functional interactions in brain networks, possibly leading to epilepsy. In **Chapter 4.2** functional connectivity and network topology in brain tumor patients with epilepsy were studied over time. Increased theta band connectivity appeared to be related to a greater number of seizures in brain tumor patients directly after neurosurgical intervention. Earlier studies are in line with these results. Increased theta band functional connectivity seems to be a pathologic feature for both epilepsy (see chapter 4.1) and brain tumors.

These findings bring up thoughts about possible mechanisms of epileptogenesis in brain tumor patients. It has been proposed that molecular changes lead to changes in functional network properties. In **Chapter 4.3** we therefore investigated the correlation between protein expression and local network connectivity. The expression of SV2A, Polyglycoprotein (P-gp) and glutamate decarboxylase (GAD) isoforms 65 and 67, which are all related to neuronal excitability, were investigated and correlated with whole-brain network topology. High expression of SV2A appeared to be related to higher connectivity between various clusters in the tumor area. This increased connectivity may be important for the development of seizures. Higher P-gp expression was related to lower between-module connectivity, and to a higher number of seizures. These results are in line with earlier research showing that an increased P-gp expression is related to high seizure proneness. There were no consistent associations between GAD65/67 expression and network topology. GAD65/67 is an enzyme that synthesizes GABA from glutamate. However, the association between GABA and epilepsy is complex, since expression of GAD65/67 increases during seizures, while lower GAD 65/67 expression is related to increased vulnerability of the brain for the development of epilepsy. This may have masked consistent associations with network topology.

The findings regarding SV2A and P-gp suggest that molecular changes may indeed influence connectivity and network topology, which ultimately leads to epilepsy. Of course, it is also possible that the alterations of local network topology are induced by seizure activity instead of the other way around.

**Treatment of epilepsy in brain tumor patients**

In the previous sections, parts of epileptogenesis in non tumorous lesions and in brain tumors were elucidated. The identification of proteins (SV2A and ADK) that contribute to tumor-related
epilepsy in chapters 2, 3 and 4, provides targets for antiepileptic drugs. The new insight in these pathways can be used to re-evaluate drugs or to develop new drugs that specifically act on these targets. Furthermore, it can be evaluated if these proteins can predict efficacy and tolerability of antiepileptic drugs, thus facilitating the choice of drug treatment (see chapter 5.3). The research described in the following section focused on the improvement of antiepileptic drug treatment in brain tumor patients.

The effect of levetiracetam monotherapy on cognitive functioning in a group of glioma patients was investigated in Chapter 5.1. In the first year of treatment no significant changes in cognitive functioning in glioma patients with epilepsy on levetiracetam monotherapy were found. Cognitive dysfunction is often the result of the cumulative effects of existing brain damage due to the tumor itself and on top of that due to therapy. It is hard to separate the effects of levetiracetam from these tumor-related factors. On the other hand the negative impact of these cumulative effects could have obscured the potentially positive effects of levetiracetam that have been described in literature.418

Older anti-epileptic drugs are believed to negatively affect cognitive functioning to a greater extent than newer anti-epileptic drugs. Therefore, in chapter 5.2 the effect of phenytoin, valproic acid and levetiracetam on cognitive functioning in high-grade glioma patients was evaluated. To avoid the difficulties in distinguishing the effects of existing brain damage due to the tumor itself, anti-tumor therapy and the antiepileptic drug, a group of high-grade glioma patients not using antiepileptic drugs was included for comparison. Patients using levetiracetam performed better than patients not using antiepileptic drugs with respect to encoding and memorization of verbal information. Patients on older antiepileptic drugs did not perform worse than patients without antiepileptic drugs, but it seems that differences between the two older antiepileptic drugs investigated in this study (valproic acid and phenytoin) were responsible for the latter findings; a positive effect of valproic acid on verbal memory probably outbalanced the negative impact of phenytoin on cognition. These findings provide arguments to consider prescribing either levetiracetam or valproic acid to epileptic high-grade glioma patients, especially to those who already have memory complaints.

Now that we further established that levetiracetam is indeed well-tolerated with respect to side-effects and cognitive functioning, it seems appropriate to prescribe levetiracetam to every brain tumor patient suffering from epilepsy. However, not all patients are seizure free on levetiracetam and no clinical parameters exist that predict the efficacy of an anti-epileptic drug. In view of the fact that SV2A is the binding site of levetiracetam and SV2A seems to be involved in epileptogenesis (chapter 2.1 and 3.1), we investigated whether SV2A expression predicts the efficacy of levetiracetam in Chapter 5.3. Glioma patients suffering from epilepsy who respond to levetiracetam showed stronger SV2A expression in tumor and peritumoral tissue than patients who do not respond: consequently SV2A expression appeared to be a predictor of the efficacy of levetiracetam. This is – as far as we know – the first time such a predictor is demonstrated. Especially when combining these results with the results of chapter 3.1, one can speculate that levetiracetam modulates the clinical effects of SV2A and restores the ability of a neuron to regulate its neurotransmitter release. Reduced SV2A expression interferes with the efficacy of levetiracetam. These results suggest that SV2A expression might be helpful in deciding whether a patient should be treated with levetiracetam. This is the first step towards personalized medicine by making a choice for the most effective drug for an individual patient based on a molecular marker.
Implications for future research

The clinically most relevant finding of this thesis is that SV2A expression might serve as a predictor of efficacy of levetiracetam, which we also showed to be an effective and well-tolerated antiepileptic drug in primary brain tumor patients. Such a correlation between expression levels of a protein known to be involved in epileptogenesis and the efficacy of a particular antiepileptic drug has not been shown before, and opens new perspectives; as many patients with primary brain tumors suffer from treatment-refractory seizures, and tumor and peritumoral tissue of almost all of these patients is available, personalized medicine based on molecular markers might be within reach.

Future research should therefore be aimed at developing personalized anti-epileptic treatment for primary brain tumor patients suffering from epilepsy. The first step should be to further establish the value of SV2A expression as a predictor for the response to levetiracetam treatment. For that purpose, the results of our study should be confirmed in randomized controlled studies. Apart from that, possible predictors for efficacy of other anti-epileptic drugs should be evaluated in order to facilitate the choice in antiepileptic drugs. Existing drugs could be re-evaluated for this matter.

Secondly, although in this thesis we have further established SV2A and ADK as important factors in epileptogenesis in primary brain tumor patients, many questions still have to be answered. Future research should be aimed at further unraveling the underlying mechanisms through which alteration of expression levels, or functional changes of SV2A and ADK play a role in epileptogenesis. It is also not clear whether a single pathway or multiple pathways need to be disturbed in order to develop seizures and what other factors are involved, such as inflammatory cytokines. Illustrative in this perspective might be that we recently showed that the inflammatory cytokine IL-1β induces alterations in the expression of Kir 4.1., which is an inwardly rectifying potassium channel 4.1. that is considered to be a key player in potassium homeostasis. A defect in the ability of astroglia to buffer extracellular potassium during high neuronal activity has been suggested to play a role in the generation of epileptic discharges. Eventually, in the case of newly found markers for efficacy, their value will have to be assessed in clinical studies. Furthermore, the identification of new markers, such as ADK, can be used as targets in the development of new antiepileptic drugs. In the case of ADK, development of antiepileptic drug treatment could even serve the dual benefit of antiproliferative and anticonvulsant therapy.

Thirdly, the underlying mechanisms through which changes in protein expression and/or function eventually lead to seizures should be further explored. Our results for the first time show a correlation between expression of SV2A and other proteins, and changes in functional connectivity and neural network characteristics. This suggests a cascade of events caused by a brain tumor leading to altered expression levels of proteins, amongst others SV2A that eventually results in functional neural network changes and epilepsy. However, in order to gain more insight basic studies are needed. Moreover, it is not yet clear whether removing the epileptic focus (either by resection, irradiation or chemotherapy) or the prescription of antiepileptic drugs may actually improve network characteristics. One could imagine that removing the tumor results in removing dysfunctional proteins with subsequently recovering of functional connectivity. Assessment of correlations between specific neurosurgical interventions, antiepileptic drug treatment, changes in seizure frequency and network alterations will result in more insight in the effects of focal treatment on network changes and seizure outcome.