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Effect of the phytocannabinoids THC and CBD in neural cell cultures under glycolytic shortage

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Johanna Palmer

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Abstract - Deutsch

Die Wirkung von Cannabinoiden in der Pathogenese von neurodegenerativen Erkrankungen, wie Parkinson, werden diskutiert. Oxidativer Stress und die damit verbundene mitochondriale Schädigung sind die beiden bedeutsamsten pathogenen Mechanismen der Parkinson Erkrankungen (PD). Cannabidiol (CBD) und Tetrahydrocannabinol (THC) können die Mitochondrien in einen Ruhezustand versetzen und somit Neurone schützen. CBD und THC werden von PD-Patient*innen teilweise zur Linderung von motorischen Symptomen genutzt. Ob eine zusätzliche Hemmung der Glycolyse, beispielsweise durch eine Low Carb Diät, Auswirkungen auf CBD/THC konsumierende Patient*innen hat, muss untersucht werden. Aus diesem Grund habe ich in meiner Arbeit die Wirkung von THC und CBD unter partieller Hemmung der Glycolyse in Primärzellkulturen und N18TG2 Neuroblastom Zelllinien untersucht. Primärzellkulturen stellen aufgrund des hohen Anteils an vulnerablen, von PD stark betroffenen dopaminergen Neuronen ein optimales Model für die Parkinson Erkrankung dar. Die Zellenkulturen wurden für diese Versuche 48h mit 10 mM 2-Desoxyglucose (2-DG) und entweder CBD oder THC in Konzentrationen zwischen 0.01µM und 10 µM behandelt. CBD in geringen Konzentrationen und 10 µM THC verringerten die metabolische Aktivität und die ATP-Konzentration in Primärzellkulturen. CBD steigerte die Überlebensfähigkeit von dopaminergen Neuronen deutlicher als THC.

CBD ist aufgrund der einschränkenden Wirkung auf die mitochondriale Aktivität bekannt dafür toxisch auf Krebszellen zu wirken. Krebszellen können ihren Metabolismus auf die Glycolyse umstellen, wenn ein Energiedefizit vorliegt. Dieser sogenannte Warburg-Effekt wurde ebenfalls in den verwendeten Neuroblastomzellen beobachtet. In den oben beschriebenen Versuchen wurde untersucht, wie die Hemmung der mitochondrialen Aktivität und die Glycolyse auf Krebszellen wirkt. Es konnte eine verstärkte Toxizität von CBD nachgewiesen werden.

Abstract - English

The effect of cannabinoids in the pathogenesis of neurodegenerative diseases, like Parkinson disease (PD) is discussed. Oxidative stress and mitochondrial impairment are the two most significant pathogenic mechanisms of PD. Cannabidiol (CBD) and tetrahydrocannabinol (THC) induce mitochondrial resting state and thus protect neurons. CBD and THC are used by PD patients to alleviate motoric symptoms. Whether an additional inhibition of glycolysis by e.g. a low carb diet influences CBD/THC consuming patients needs to be investigated. Therefore, I investigated the effect of THC and CBD under partial inhibition of glycolysis in primary neural cell cultures and N18TG2 neuroblastoma cell lines. Primary cell cultures represent an optimal model for PD due to the high expression of vulnerable dopaminergic neurons severely affected by PD. For these experiments, cell cultures were treated for 48h with 10 mM 2-deoxyglucose (2-DG) and either CBD or THC at concentrations between 0.01 µM and 10 µM. Low concentrations of CBD and 10 µM THC decreased the metabolic activity and ATP concentration in primary neural cell cultures. CBD increased the survival of dopaminergic neurons more markedly than THC.

CBD is known to exert toxic effects on cancer cells due to its mitochondrial inhibition. Cancer cells can change their metabolism to glycolysis when they are in an energy deficit, called ‘Warburg effect’. This Warburg effect was also observed in our N18TG2 neuroblastoma cells. In the experiments described above, the effects of CBD and 2-DG on cancer cells were investigated. An increased toxicity of CBD could be demonstrated.

1 Introduction

1.1 The Endocannabinoid System

In vertebrata and evertebrata, except insects, the endocannabinoid system (ECS), which consists of cannabinoid receptors, endogenous ligands and catalyzing and hydrolyzing enzymes, regulates multiple physiological processes of the body including antinociception, perception, mood, the cardiovascular and immunological system, memory, and reward (1,2).

1.1.1 Endocannabinoids

Endocannabinoids are the endogenous ligands of the ECS triggering the cannabinoid receptor signaling cascade. Unlike to other, by the majority hydrophilic neurotransmitters, like acetylcholine or adrenaline, endogenous ligands of the endocannabinoid system are lipophilic and synthesized on demand from arachidonic acid (an essential fatty acid) by the five main enzymes of the ECS: *N*-acyl-phosphatidyl-ethanolamine-selective phospholipase D (NAPE-PLD) and α- and β- diacylglycerol lipase (DAGL). Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) function as hydrolyzing enzymes. In addition to these five main enzymes, other biosynthesizing and inactivating enzymes are important to complement this system (3).

The two most important endocannabinoids are 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamide (anandamide) (3). 2-AG is a full agonist for CB1 and CB2 receptors and shows a lower affinity to them than anandamide does. As a partial but high-affinity agonist for CB1 and CB2 receptors, anandamide inhibits 2-AG activity (1).

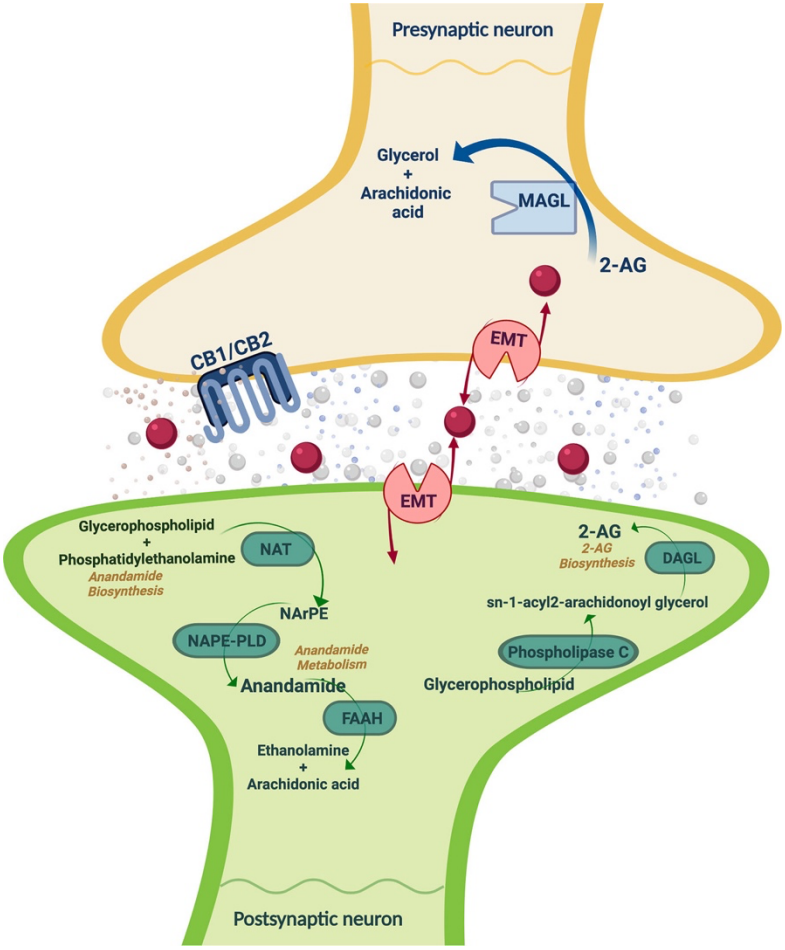

Figure 1: Function and components of the ECS. adapted from ((4)**)** Cannabinoids are synthesized postsynaptically and released to the synaptic cleft. There, they bind on presynaptic cannabinoid receptors, resulting in a decreased release of neurotransmitters. 2-AG, 2-arachidonoylglycerol; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; DAGL, EMT: endocannabinoid membrane transporter; NAT, N-acyl transferase; NArPE, N-arachidonoyl phosphatidylethanolamine; NAPE-PLD, N-acylphosphatidylethanolamine specific phospholipase D; DAGL, diacylglycerol lipase (4).

1.1.2 CB1 and CB2 – Receptors

Endocannabinoids have two receptors as sites of action - CB1 and CB2. Both are G protein coupled receptors (GPCRs), which are distributed throughout the whole organism and perform different mechanisms of action (5).

CB1 receptors are mainly expressed throughout the brain. A high density can be found in the hippocampus, basal ganglia, and cerebellum. According to their antinociceptive function and effect on motor coordination, CB1 receptors are strongly expressed in the dorsal primary afferent spinal-cord regions and to a minor extent in the brainstem (6). CB1 receptors are also

found on neurons in the peripheral tissue. This includes the urinary bladder, gastrointestinal tract, heart, vessels, uterus, spleen, testis, lung, and thymus (7).

CB2 receptors are primarily located in the immune and hematopoietic systems and only in small amounts in the CNS. They can control cytokine releases and have main impact in the regulation of inflammatory processes. CB2 receptors could also be found in the brain, on astrocytes and microglia, blood vessels and some neurons. Due to these localizations, in the peripheral and central nervous system, CB2 receptors can effectively participate in proliferation, differentiation and survival of neuronal and non-neuronal cells (2).

CB receptors perform retrograde signaling from their presynaptic localization on GABAergic, glutamatergic, dopaminergic, and other neurotransmitter neurons (9). Endocannabinoids are synthesized at the postsynaptic membrane. They pass retrogradely across the synaptic cleft to the presynaptic membrane, where they bind to the CB1 or/and CB2 receptors (9). For this retrograde transport the endocannabinoid gets supported by the fatty acid binding protein (FABP) (10). Once the receptor is activated, the K^+ inflow increases and lead to a hyperpolarization. As a result of this, Ca^{2+} channels cannot be opened, and the transmitter vesicle fusion cannot be activated. This mechanism controls the distribution of neurotransmitter into the synaptic cleft. Since endocannabinoids are produced only as required, the effect can be strongly limited locally and temporally (9). Additionally, the neuromodulative effect of endocannabinoids plays a role in long-term synaptic depression (11).

Like GPCRs in general, the membranous cannabinoid receptors can inhibit the production of adenylate cyclase, which prevents the conversion of adenosine monophosphate (AMP) to cyclic AMP (cAMP), which leads to the inhibition of the signal cascade influencing the synaptic transmission (2).

1.1.3 Phytocannabinoids

Cannabinoids are divided into 3 main groups: Endocannabinoids, synthetic cannabinoids and phytocannabinoids. Phytocannabinoids are extracted from the plant *Cannabis sativa*. To the state of knowledge in 2016, 142 phytocannabinoids, which consists of C21 terpene phenolic compounds, could be extracted so far (9). In their basic acidic form, they can be found at two different locations in the cannabis plant: the resin and the bast cells (12).

Phytocannabinoids have previously been described as potential therapeutics for neurodegenerative diseases such as Parkinson Disease (PD), Alzheimer Disease (AD), Amyotrophic Lateral Sclerosis, Autism Spectrum Disorders and Epilepsy. One cannabonid in particular, CBD, has been repeatedly brought into focus (13).

Cannabidiol (CBD)

CBD, the main cannabinoid of fiber hemp, is a non-psychoactive 21-carbon terpene phenolic compound with numerous molecular targets. Its therapeutic potential is based on neuroprotective, cardioprotective and anti-inflammatory effects (14).

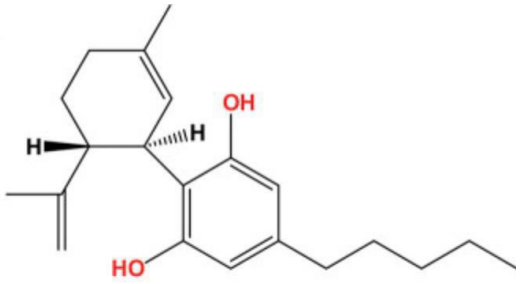

Figure 2: Structural formula of Cannabidiol. (Adapted from (15))

Furthermore, CBD is a low affinity agonist on CB1 and CB2 receptors (16). The binding on CB1 receptors mainly causes indirect effects. A much smaller concentration of CBD is needed to antagonize CB1 agonists like for example WIN55212 and CP55940 than to activate a CB1 receptor (14). Likewise, CBD inhibits anandamide hydrolysis by FAAH by an indirect increase of anandamide levels (3,9). CBD's binding at the CB2 receptor can suppress inflammatory mechanisms (17).

This immunosuppressive effect of CBD is shown by its ability to drive endogenous adenosine transduction by [^{3}H]thymide uptake into microglial cells, inhibiting its uptake into DNA and decreasing the uptake of [^{3}H]adenosine. Furthermore, CBD can bind to other receptors than CB1 or CB2. For instance, CBD acts as an agonist of the 5-hydroxytryptamine receptor 1A by antagonizing its agonist serotonin. CBD also acts as transient receptor potential channel (TRPV2) agonist. As unselective cation channels, they have an important function in nociception (2).

Tetrahydrocannabinol (THC)

THC is known as the main psychotropic component and major constituent of the drug type *Cannabis sativa* plant (drug hemp).

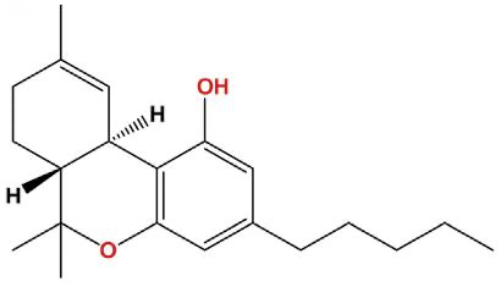

Figure 3: Structural formula of Tetrahydrocannabinol (Adapted from (15))

THC acts as partial agonist on CB1 and CB2 receptor. Cannabinoid receptor expression levels and signaling efficiency, as well as endogenous cannabinoid release, influence the expected response of THC (18).

Various therapeutic applications are attributed to THC. Due to its ability to suppress proinflammatory mechanisms and chemokine production, THC can be used to treat chronic inflammatory diseases. In addition, it is considered to be neuroprotective (17). Since the CB1 receptors are largely found on presynaptic terminals of glutamatergic and GABAergic synapses, THC mediates the suppression of excitatory and inhibitory neurotransmitters. Therapeutic success was achieved with THC, because it doesn't block the glutamatergic synapse activity completely and therefore produces no detectable side effects (2).

1.1.4 Effect of Phytocannabinoids in Neurons

Neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington disease (HD) and amyotrophic lateral sclerosis and research on their treatment options are becoming increasingly important in our society. Our society is aging progressively due to increasing life expectancy. As a result, the population group with a high risk of developing neurogenerative diseases will continue to grow significantly (19).

Neurodegenerative diseases are characterized by oxidative stress, neuroinflammation, mitochondrial impairment (20) and excitotoxicity (21).

Neurodegenerative diseases increase the release of excitatory glutamate by the cystine/glutamate antiporter, which leads to oxidative stress and excitotoxicity-induced cell death. Likewise, oxidative stress elevates reactive oxygen species (ROS), which is associated with the damage of DNA, lipids, and proteins (22). Neuroinflammation is characterized through microglia and astrocyte activation and the decrease of inflammatory cytokines in the CNS (23). Mitochondrial impairment occurs due to defects in the process of oxidative phosphorylation, enhanced oxidative stress and damaged calcium transport (24). The next chapter explains the interconnection between the pathological pathways and gives information about the protective effect of THC and CBD in neurodegenerative diseases.

Alzheimer´s disease

Alzheimer´s disease (AD) is the most common disease of the CNS, as well as the most common form of dementia (60%). The disease is characterized by memory impairment, loss of the neurocognitive functions such as communication, orientation, and perceptual skills.
Two main pathological changes explain the pathogenesis of the disease ever since Alois Alzheimer discovered AD in 1907 (19): positive lesions due to accumulation of neurofibrillary tangles (NFTs) and senile plaques (SP). Due to the amyloid stores, the content of amyloid-β-42 (Aβ42) protein within the cerebrospinal fluid decreases. In addition, increments in tau protein and phosphorylated tau protein can be recognized within the cerebrospinal fluid. The other pathological change is explained by negative lesions due to synaptic and neural loss. This synaptic damage in the limbic system and neocortex explains the partial amnesia in the initial stage of AD. Mitochondrial damage, oxidative stress, and defects on axonal transport are mechanisms of synaptic loss, which can lead to a loss of dendritic spines, presynaptic terminals, and axonal dystrophy (19,25,26). Additionally, excitotoxicity has been linked to AD pathogenesis in several studies. By activating NMDA receptors through amyloid-β peptide, ROS-induced neurodegeneration is triggered. AD's pathological features can be reduced by inhibiting NMDA receptor-mediated transmission, e.g. by NMDA receptor antagonists, like memantine, which have also been shown to have a good therapeutic impact on symptoms of AD patients (25).
The effect of the endocannabinoid system, especially the synaptic responsiveness and plasticity are being discussed as a potential approach for therapeutic use.
THC, as a modulator of the endocannabinoid system, is discussed to have some possible proactive mechanisms associated with AD (27). An *in vitro* study showed an anti- amyloid-β-

aggregation activity of THC. In this context, it also stimulates the removal of intracellular amyloid-β and blocks the inflammatory immune response (28). The effect of THC on neurotransmitters has been demonstrated by potent inhibition of acetylcholinesterase, compared to already known therapeutics. Furthermore, THC inhibits the release of high levels of glutamate and thus counteracts excitotoxicity and oxidative stress. The addition of CB1 antagonists did not prevent this effect, leaving the mechanism of action of THC on excitotoxicity further unclear (13). Nonetheless, the greatest neuroprotective effect of THC currently described is provided by THC acting at the CB1 receptor. Increased CB1 activity results in increased release of BDNF factors, which regulate morphological and physiological synaptic plasticity. Author showed that CB1 activity induced BDNF expression contributes significantly to neuroprotection (29).

Treatment options for AD using CBD are still under investigation. So far, CBD has been known to have effects against glutamate-induced toxicity in primary mesencephalic cell cultures (13). The tau protein hyperphosphorylation plays an important role in the pathogenesis of AD as described above. CBD inhibits the beta amyloid-induced production of tau protein hyperphosphorylation nitric oxide (30).

Furthermore, CBD suppressed the gene expression of AD-related genes such as the beta and gamma secretase genes in mesenchymal stem cells (31).

The neuroinflammatory effect of CBD has also been observed in AD patients. In an *in vivo* model of amyloid ß induced neuroinflammation, CBD caused a suppression of proinflammatory glial molecules in the hippocampus (27).

Huntington disease

Huntington disease (HD), a dominant autosomal neurodegenerative inherited disease occurs predominantly in 5.5 of 100,000 people around the age of 30 in Europe. HD is characterized by the loss of motion such as myoclonus, dyskinesia and bradykinesia, the loss of cognitive capacity and psychosomatic symptoms due to the atrophy of basal ganglia and cerebral cortex (19).

Neuropathologically, HD underlies a mutation of the huntingtin gene (HTT gene). This gene consists of 27 repetitions of glutamine encoding cytosine-adenine-guanine-triplet (CAG). Due to this mutation, the number of CAG strung together is increased and elongated the polyglutamine tract (35 copies). Mutated HTT genes are cell toxic and highly expressed in neurons, which may explain neurological symptoms of the disease. Mitochondrial impairment

is also discussed to emphasize the pathogenesis of HD. A decrease of the activity of complexes II, III and IV of the mitochondrial chain and defects of the Ca^{2+}-dependent mitochondrial enzyme aconitase, which occurs in the striatum of HD patients, could be demonstrated (23). This influences on mitochondrial activity may also be due to the presence of the expanded N-terminal fragments of HD, whereby the mutant polyglutamine chains can directly bind to proteins influencing the mitochondrial division and axonal transport.

Furthermore, an increase of markers for oxidative damage in DNA and a decrease of the immunoreactivity of cytochrome-based proteins has been showed.

Neuroinflammation is characterized through the expression of cytokines and activation of microglia, which have been demonstrated as an early sign in the premanifest stage of HD. The extent of glial activation correlates closely with the age at which HD will clinically occur, as determined by CAG repeats. The hypothesis is that the polyglutamine tract may influence the transcriptional activity of some proinflammatory genes in microglial cells. Mutant polyglutamine-containing fragments of huntingtin in the extracellular space may also directly trigger aseptic inflammation in the extracellular space. Closely related to the development of neuroinflammation in the brain in HD is oxidative stress induced by mutant huntingtin. HD patients have pathological iron deposits in their brains and therefore induce reactive oxygen species (ROS), as well as the huntingtin protein that is controlled by ferrous iron. Thus, increased oxidative stress can be assigned to HD as a neuropathological effect.

Moreover, HD shows an activation of N-methyl-D-aspartate (NMDA) receptor of glutamate, whereby more glutamate is transported into the cell, which leads to excitotoxicity and an increase in Ca^{2+} in neurons. The glutamate excitotoxicity is associated with mitochondrial impairment and oxidative stress on the one hand. One the other hand, it decreases the levels of glutamate transporter GLT-1/S1c1a2. As a result, less glutamate can be transported out of the cell and thus no protection can be guaranteed for the affected neuron (21,23).

HD is simulated trough different animal models to mimic various neuropathological pathways: R6/2 mice with a genetic deficiency in CB1 receptors, 3-nitroproprionate-lesioned mice to mimic mitochondrial damage, oxidative stress and calpain activation, and malonate-lesioned rats to mimic neuroinflammation. THC attenuated the loss of striatal neurons in R6/2 mice models by CB1 receptor activation (32), while CBD showed neuroprotective effects in 3-nitroproprionate-lesioned mice models, but controversy was inefficient in malonate-lesioned rat models (33).

Both, CBD and THC as ligands of CB2 receptors, displayed neuroprotective properties by suppression of inflammatory events and microglial activation. Treatment with a combination of

CBD and THC preserved striatal neurons in 3-nitroproprionate-lesioned mice models and malonate-lesioned rat models, but not in R6/2 mice models. In this model, only treatment with THC displayed neuroprotective effects (34).

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, which occur in 10-12/100,000 people per year in Europe. There are three factors that can trigger this disease: genetic, environmental, and age-related factors. ALS affects the upper motoneurons (UMN) and lower motoneurons (LMN) and subsequently leading to a progressive muscle weakness. After the onset of the first symptoms, the affected person still has about 2-5 years until the respiratory musculature is also involved and vital mechanisms can no longer be carried out. Additionally, non-motor symptoms such as speech problems, coordination difficulties and character changes occur (35).

Neuropathologically it is characterized trough a loss of synaptic connections in neurons by axonal retraction and the loss of neuromuscular connection leading to cell death of LMN and UMN. In addition, the DNA and RNA-binding protein TDP-43 could be verified in inclusions of the surviving neurons. Physiologically its mainly located in the nucleus but found misplaced in the cytoplasm leading to a formation of cytoplasm aggregates and phosphorylation of this protein.

Up to now, several pathogeneses have been hypothesized as the cause of ALS: oxidative stress, failure of proteostasis, mitochondrial impairment, neuroinflammation, excitotoxicity, defects in nucleocytoplasmic transport and axonal transport, oligodendrocyte dysfunction, cytoskeletal malformation, RNA metabolism disturbance and failure of DNA repair.

Beneficial effects of THC on SOD-1 (superoxide dismutase) mutant mice could first be proven in 1990. The new model of ALS (transgenic mouse model of TDP-43) could display an activation of CB2 receptors and therefore improved motor behavior, reduced glial activation and preserved spinal motor neurons. Activation of the CB1 receptor also reduces, albeit to a lesser extent, glial activation (36). Non-receptor mediated mechanisms by CBD and THC are antioxidant effects, which can be associated with PPAR gamma signaling, since PPARs regulate the ß-oxidation in peroxisomes. The combination of both cannabinoids, CBD and THC could not elicit neuroprotective mechanisms or prolong the life of the SOD-1 postsymptomatic mutant mice (34).

Parkinson disease

Parkinson disease (PD), known as a chronic progressive neurodegenerative disease occurs in 1% of people over the age if 65 years (19). It is clinically characterized through mainly motoric symptoms including bradykinesia, resting tremor, postural instability and rigidity, as well as sensorics, vegetative, psychological, and cognitive symptoms.

Neuropathologically PD shows a degeneration and loss of dopaminergic neurons in the substantia nigra pars compacta, whereby 60-70% of the neurons already have been damaged before the first symptoms of PD occur (37).

The intraneuronal cytoplasmic inclusion bodies, called Lewy bodies, detected in PD patients, also lead to premature death of the affected neurons. The Lewy bodies consist of proteins, mainly alpha synuclein, ubiquitin, and modified cytoskeletal components. Such protein deposits are not specific to PD, but are found in many neurodegenerative diseases, and the exact neuropathological role of Lewy bodies has not yet been fully elucidated (21).

Until now, several pathogeneses have been hypothesized as the cause of PD: oxidative stress, mitochondrial impairment, neuroinflammation, excitotoxicity, and apoptosis. Due to the oxidative metabolism of dopamine, the metabolites peroxide, hydroxyl radicals and ROS can induce oxidative stress. A reduction of the main brain antioxidant glutathione and an increase of iron levels (pro-oxidant) could also be demonstrated.

Mitochondrial impairment in PD is discussed to be caused by defects in complex I of the respiratory chain in about 40% of cases, but also by inhibition of levels of alpha ketoglutarate dehydrogenase (24).

Another pathogenic pathway is formed by neuroinflammation by activating microglia cells, increase cytokine distribution and upregulation of inflammatory factors in the striatum and cerebrospinal liquid. Anti-inflammatory agents, like minocycline, can block glial activation and cytokine production and therefore protect neurons against oxidative stress and damage by this factor (37). Glutamate-induced excitotoxicity is discussed to occur secondarily in PD patient due to mitochondrial impairment and a defect in NMDA receptors (37).

1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP), its metabolite 1-methyl-4-phenylpyridinium (MPP^+), rotenone and 6-hydroydopamine (6-OHDA) are toxin-induced PD models, which showed an increased vulnerability of neurons to excitotoxicity and oxidative stress by stimulation of the glutamate efflux from neurons. In contrast to this, antagonists of the NMDA receptor reduce the efficacy of MPP^+ (38).

Potential effects of CBD and THC in Parkinson's disease models could be due to the presence of CB1 receptors in the substantia nigra. As a retrograde messenger of the glutamatergic and GABAergic system, phytocannabinoids interact with the dopaminergic system as well. CB1 receptor mediated inhibition of GABAergic inhibition explains phytocannabinoids excitatory effect in the substantia nigra. A feedback mechanism is created between the dopaminergic system and the striatum (target region).

Numerous studies have shown that CBD and THC are used as external neuroprotectants and operate by inhibiting glutamate transmission and thus protecting dopaminergic neurons affected by oxidative stress (13,17). Nevertheless, the impact of CBD on the dopaminergic system is discussed controversially. Murillo-Rodríguez et al. 2011 demonstrated that CBD increases the distribution of dopamine. In this experiment the dopamine levels in the nucleus accumbens, part of the ventral part of the striatum, increased for three hours after injecting CBD into the lateral hypothalus (39).

CBD could induce an attenuation of the dopaminergic activity in the ventral tegmental segment (40). Based on these findings, CBD and THC may prevent neurodegenerative effects in cell culture models for PD.

The Neurochemistry Group of the VetmedUni Vienna is working with cell culture models for PD. Primary mesencephalic cultures represent an optimal model to test the effects of different drugs on pathogenicity due to the relatively high content of vulnerable dopaminergic neurons, which degenerate during the pathogeneses of PD.

This working group could demonstrate that THC increases the viability of dopaminergic neurons, while CBD decreases the viability of dopaminergic neurons and hereby shows neurotoxic properties in primary mesencephalic cultures. However, a protective effect of THC and CBD against MPP+-induced oxidative stress could be found, where THC showed a more pronounced neuroprotective effect than CBD (8).

Another study of the working group gave evidence that CBD potects neurons against stressful conditions and neuronal cell death via the heme oxygenase (HO) system, playing a key role in oxidative stress. Rotenone, a complex I inhibitor of the mitochondrial respiration chain was used to perform this experiment in primary mesencephalic cell cultures and N18TG2 neuroblastoma cells. The toxic effect of rotenone was partly reversed by CBD, but not in THC. Furthermore, THC and CBD act as inhibitors of the HO activity, whereby CBD is more potent. According to (41), the efficacy of CBD as anticancerogenic agent seems to work also via the restriction of mitochondrial function by reducing the mitochondrial respiration on complex I and

II. THC and CBD decrease mitochondrial function, which is proposed to be a protective mechanism against cellular stress.

1.2 Energy Metabolism of the Cell

Cell metabolism is based on the conversion of macronutrients into biochemically available molecules. For energy metabolism, the main energy equivalent is ATP. Lipids, carbohydrates, and their degradation products are used to obtain ATP in the mitochondria of cells. Glycolysis, citric acid cycle and oxidative phosphorylation are the pathways which generates 36 units of ATP out of one molecule glucose (42).

Glycolysis is a catabolic process in which one molecule of glucose is converted in two molecules of pyruvate. In this process, two molecules of ATP and two molecules of NADH are obtained as energy equivalent. Depending on whether glycolysis is aerobic or anaerobic, the consumption of the resulting pyruvate differs.

In aerobic glycolysis, the produced pyruvate is transferred from the cytoplasm into the mitochondria, where it is converted into acetyl-CoA by oxidative decarboxylation by the pyruvate dehydrogenase complex. This acetyl-CoA is incorporated into the citric acid cycle (CAC), transported into the mitochondrial matrix, and further oxidized. In contrast, during anaerobic glycolysis, pyruvate is reversibly reduced to lactate by lactate dehydrogenase (LDH) under NADH consumption.

Acetyl-CoA is the initiating molecule in the citric acid cycle and is thereby subjected to the oxidative carboxylation. In this multi-step process, three $NADH+H^+$, one $FADH_2$ and one GTP are obtained as energy and further used for oxidative phosphorylation to obtain ATP with H_2O as a by-product (42).

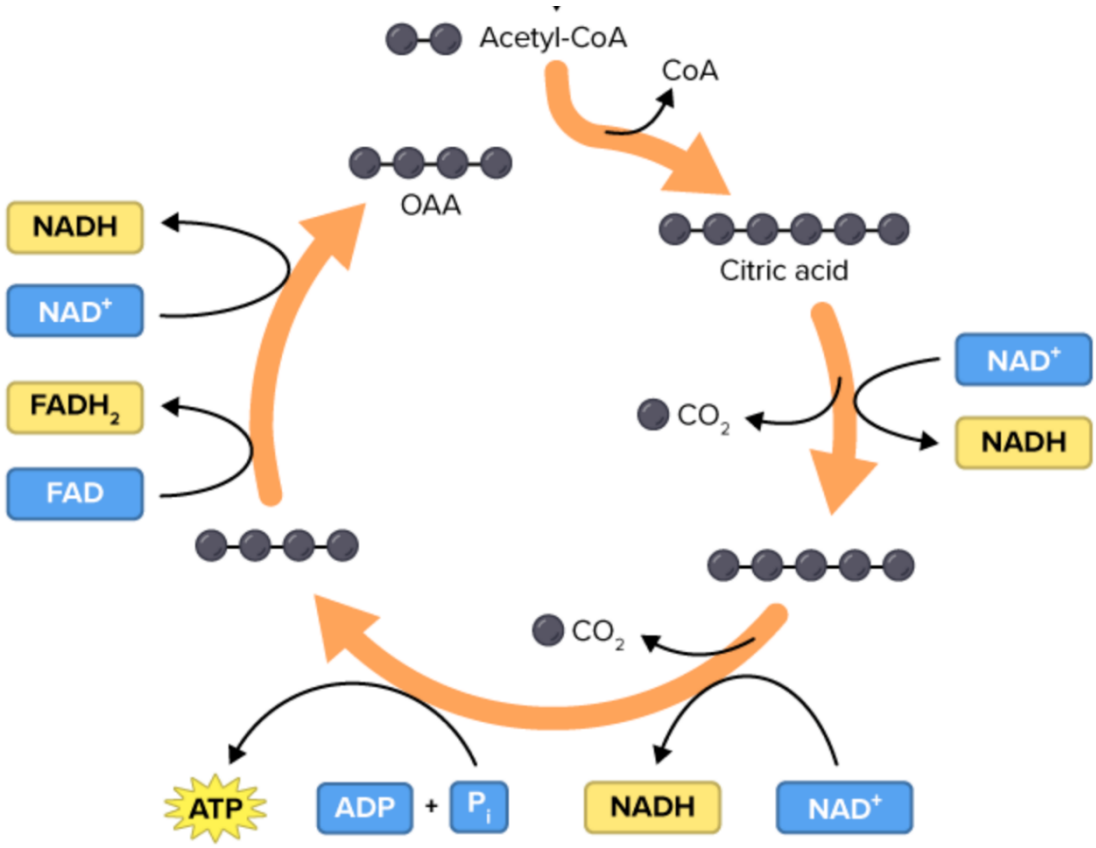

Figure 4: Overview of the citric acid cycle with focus on the formation of energy equivalents. The oxidation of Acetyl-CoA to ATP is carried out with the intermediate metabolites GDP, NAD^+ und FAD and $C0_2$ as a by-product (43).

The process of oxidative phosphorylation basically consists of the delocalization of electrons from the reduction products $NADH+H^+$ and $FADH_2$. These electrons are transported over four protein complexes by the electron transport chain in the mitochondrial membrane to bind the oxygen present in the mitochondrial matrix. The energy gained by this exergonic redox reaction is used to build up a proton gradient across the mitochondrial membrane and thereby pump H^+-ions into the mitochondrial matrix, which produces energy. This energy is used to produce ATP (42).

1.2.1 Energy Metabolism in Cancer Cells

Glucose is the most important source of energy for every cell. As described above, energy production from glucose proceeds via three pathways: Glycolysis, citric acid cycle and oxidative phosphorylation. Of these three, glycolysis is the most inefficient one (44).

According to the scientist Otto Warburg, the glucose metabolism of a cancer cell changes from oxidative phosphorylation to aerobic or anaerobic glycolysis. Under oxygen-deficient conditions, cancer cells can generate high amounts of energy, compared to the surrounding tissue through anaerobic glycolysis and thus use the lactate produced as an energy source (45). During the multi-step process of glycolysis, one molecule of glucose turns into two molecules of pyruvate, which are further fermented into lactate.

Even though the turnover of glycolysis (two ATP) is much lower than for oxidative phosphorylation, the tumor tissue can compensate with a typically increased expression of glucose transporters (GLUT) and glycolytic enzymes. Reasons for the conversion of this metabolism could be genetic mutations, mitochondrial modifications, and tumor hypoxia (15).

As 2-Desoyglucose (2-DG) competes with D-glucose for the uptake into the cell, numerous studies have shown that 2-DG and thus the inhibition of glycolysis is a promising anticancerogenic therapy (45,46).

1.2.2 2- Desoxyglucose – Inhibitor of Glycolysis

2-DG is a synthetic and nontoxic glucose analog, in which hydrogen replaced the 2-hydroxyl group from D-glucose.

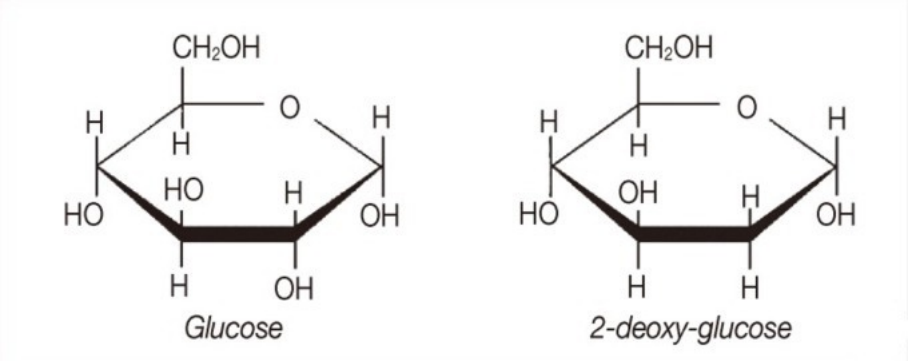

Figure 5: Comparison of the structure from glucose and 2-DG (47)

Like glucose, it enters the cell via glucose transporters (GLUT) and gets phosphorylated by hexokinase II to 2-deoxy-D-glucose-6-phophate. Because of the missing 2-hydroxyl group the 2-desoxy-D-glucose-6-phosphat is not converted into its isomer fructose-6-phospate and therefore glycolysis stops (45,46).

1.3 Cell Culture Models

In my, study, cell cultures were used to investigate the effects of THC/CBD on cells treated with 2-DG. Cell cultures require certain environmental conditions for distinctive cell growth. Temperature, CO_2 content of the environment, pH-value, osmotic pressure, and the nutrient supply must be optimally adjusted during the whole cultivation. Cells can be grown under these conditions either in different volumed flasks, culture plates, like 4/48/96 – well plates or Petri dishes and others.

Animal testing is mandatory for the development of medication, but controversially discussed for ethical reasons. In basic science, it is increasingly replaced by cell cultures, which are used as models for research in neurodegenerative diseases. Growing cells *in vitro* offers several advantages. It is easier to control the cell environment and the exact cell number for different assays. Additionally, it is cheaper, faster and avoids interactions with other physiological processes in the organism. Due to these isolated conditions, the knowledge gained must always be evaluated in an *in vivo* model, since an artificial system can always be subject to sources of error.

1.3.1 Primary Dissociated Mesencephalic Dopaminergic Cell Culture

Neurons are extracted from the mesencephalon of a 14-day-old mouse embryo to establish a primary dissociated mesencephalic dopaminergic cell culture. The young age of the used animals is decisive since the cells are still dissociable at this stage of development but are already determined as dopaminergic neurons. An advantage of primary cell culture is the preservation of neuronal morphology, diversity, and physiology of the tissue of origin. Therefore, cellular mechanisms can be traced in relation to the organism and not exclusively to the cell type.

Especially in the context of the research of PD, primary mesencephalic cell cultures offer a good basis for research due to their content of dopaminergic neurons. Primary mesencephalic cell cultures represent a heterogenous culture with about 60% of glial cells on average. Additionally, the glia/neuron ratio varies leading to variations of results. On the other hand, this type of cell culture allows a greater comparability with the *in vivo* situation.

During cultivation, the neurons grow out and develop axons and dendrites, allowing the culture to interconnect synaptically. Nonetheless, the mature cultures show lower connectivity and reduced organization than the mesencephalon in vivo.

In this thesis, murine primary mesencephalic cell cultures were used to investigate the number and morphology of dopaminergic neurons under different conditions, which accounts for less than 0.1% in the brain *in vivo* (48). The site of greatest concentration of dopaminergic neurons is situated in the brain in the substantia nigra pars compacta, which is included in primary dissociated mesencephalic cell culture. Moreover, they are very sensitive to oxidative stress and thus represent an optimal model not only for PD, but also other neurogenerative diseases in which oxidative stress occurs.

1.3.2 N18TG2 Neuroblastoma Cell Lines

N18TG2 cells are 6-thioguanine (chemotherapeutic agent) resistant neuroblastoma cells, which were extracted from the C1300 tumor of a male A/J mouse (49). The tumor cell lines proliferation time of N18TG2 is about 25 h. Cell lines in general are easy to handle, cost effective and have a high compatibility with European ethical standards. Moreover, N18TG2 cells show a stable expression of CB1 receptors, which is a basis for the research on cannabinoids.

CBD has been known for its anticancerogenic effectiveness for some time. According to that, the Neurochemistry Group also demonstrated that CBD is neurotoxic to N18TG2 cells (50). For our purposes, we are using this tumor cell lines and primary dissociated mesencephalic cells to compare the differential effect of cannabinoids in primary and cancer cells.

1.4 Aims

The aim of this study was to investigate the effects of CBD and THC under partial inhibition of glycolysis. At the Neurochemistry Group, THC and CBD were found to be neuroprotective in cell culture models of mitochondrial impairment. However, CBD was also found to be mildly neurotoxic in primary neural cell cultures. Both phytocannabinoids inhibit mitochondria activity, but CBD to a higher extent.

The use of CBD in cancer therapy has been discussed. Some cancer cells can use glycolysis as their main source of energy. A restraint ATP formation in the mitochondria of cancer cells can possibly be compensated by an upregulated glycolysis. The glycolysis inhibitor 2-DG was used to enhance the effect of CBD toxicity in cancer cells. The glycolytic shortage caused, for example, by a ketogenic diet of affected cancer patients is regarded as an effective support for cancer therapy. To make use of CBD as a cancer drug it should have a comparatively low toxicity in primary neural cell, when co-treated with 2-DG.

In our study, we wanted to study, which impact glycolytic shortage in combination with mitochondrial inhibition has. It is known that phytocannabinoids can alleviate clinical motor symptoms of PD. One aim of the study was to investigate whether the inhibition of glycolysis and concomitant treatment with cannabinoids results in degenerative effects due to energy shortage. This may be of interest for the consideration of a low glucose diet in combination with THC/CBD treatment.

Hypothesis: In cells with reduced glycolysis activity, treatment with CBD, but not THC, leads to cell degenerative effects.

The following questions should be answered in this study:

(1) Do CBD and THC affect cancer cells more than primary neural cell cultures?
(2) Does the partial inhibition of glycolysis has a greater effect on cancer cells than on primary neural cell cultures?
(3) Does co-treatment of 2-DG with CBD reveal a greater toxicity than with THC?
(4) Does CBD and THC exert neuroprotective effects on primary neural cell cultures, which have been affected by the partial inhibition of glycolysis?
(5) Are neurotoxic effects of CBD more pronounced in cancer cell than in primary neural cell cultures when glycolysis is partially inhibited?

2 Material and Methods

2.1 Material

All chemicals used were ordered from Sigma Aldrich, Germany. Exceptions were specifically characterized.

2.1.1 Animals

Pregnant OF1/SPF mice (gestation day 14) from the Institute of Laboratory Zoology and Veterinary Genetics (Austria). The study was discussed and approved by the ethical and animal welfare committee (ETK-005/01/2022) of the University of Veterinary Medicine Vienna.

2.1.2 Buffers and Solutions

Table 1: Media for primary dissociated mesencephalic cells

basic medium (BM)	N4- Medium
50 mL DMEM	50 mL DMEM
500 µL HEPES buffer (1 M)	500 µL HEPES buffer (1 M)
370 µL glucose (20% solution)	370 µL glucose (20% solution)
1 mL glutamine (200 mM)	1 mL glutamine (200 mM)
100 µL streptomycine (50 mg/mL) /penicillin (50,000 U/mL) (Roche, Germany)	
5 mL fetal bovine serum	1 mL B27 (Invitrogen, UK)

Table 2: Media for N18TG2 cell lines

maintaining-medium (MM)	treatment-medium (TM)
43 mL DMEM	47 mL colorless DMEM
5 mL fetal bovine serum	1 mL B27 supplement
1 mL Na-pyruvate (100 mM)	1 mL Na-pyruvate (100 mM)
1 mL L-glutamine (200 mM)	1 mL L-glutamine (200 mM)

2.2 Methods

2.2.1 Primary dissociated mesencephalic cells

Preparation

Pregnant OF1/SPF mice were cared and handled according to the guidelines of the European Union Council (2010/63EU) for the use of laboratory animals. The preparation of primary mesencephalic cells was performed according to Radad et al., 2004.

On gestation day 14, mice were sacrificed by CO_2 and cervically dislocated. The abdomen was opened, the uteri removed and put into a large Petri dish with Dulbecco's phosphate buffered saline (DPBS, Gibco, UK). The following dissection steps were performed under aseptic conditions. After abscission of the uteri, placentae and extraembryonic membranes were removed, each embryo was cut in half with a curved scissor right above the liver. The Petri dish with the upper parts of the embryos were put under a stereoscope, brains were cut out and ventral mesencephala excised. The meninges were removed carefully. The tissue was dissected with a scalpel and transferred in a centrifuge tube with 2 mL trypsin solution (0.05%, Invitrogen, UK). 2 mL of Hank's balanced salt solution (Gibco, UK) containing DNase (10 mg/mL) were added and incubated for 7 min at 37 °C. The trypsin reaction was stopped with 2 mL of trypsin inhibitor (0.25 mg/mL, Gibco, UK). Subsequently the tube was centrifuged for 4 min at 100 g and the supernatant was removed.

The remaining pellet was resuspended in 3 mL of prewarmed basic medium (BM) containing 60 µL DNase (10 mg/mL) and carefully triturated. After 10 min sedimentation time 2 mL of the supernatant was transferred into a flask with 6 mL prewarmed BM. This trituration step was repeated twice, whereby the first time 3 mL of suspension was removed and the second time 4 mL. An aliquot of cell suspension (20 µL) mixed with 20 µL of trypan blue (0.4%) was transferred to a Neubauer counting chamber, and cells were counted. The cell suspension was diluted to a final concentration of 750,000 cells/mL and 340 µL/well on a 48 well-plate and 150 µL/well on a black 96-well plate and a white 96-well plate were seeded on pre-coated plates (PDL (0.1 mg/mL) Gibco, UK). Cultures were incubated at 37 °C with 5% CO_2.

Cultivation

Cultures were incubated at 37 °C with 5% CO_2 and maintained for a total of 14 days. Half of the medium was replaced on the first day in vitro. On the second DIV a full medium exchange was done. On the 5th DIV, a mixture of equal volumes of BM and N4 was used for the medium replacement. From the 6th DIV on, serum free N4 medium was used for medium exchange.

2.2.2 Cultivation of the N18TG2 neuroblastoma Cell Line

The adherend growing N18TG2 cells were kept in cell culture flasks (25 cm^2), filled with 5 mL medium and incubated at 37 °C and 5% CO_2. Maintaining was performed when culture reached approximately 80% confluence (48-72 h). The Maintaining includes splitting (1:10) the N18TG2 cells and refill remaining 0.5 mL cell suspension with fresh maintaining-medium (MM). Before treatment a black 96-well plate and a white 96-well plate were pre-coated with PDL (0.1 mg/mL) and incubated at 37 °C and 5% CO_2 for 1 hour. After discarding the PDL solution and a washing step with DPBS, cells were seeded into the plates. In detail, MM was removed from the cell culture flask and the cells were washed down with 5 mL of prewarmed fresh MM. This solution is transferred to a sterile flask and an aliquot of cell suspension (20 µL) mixed with 20 µL of trypan blue (0.4%) was transferred to a Neubauer counting chamber and cells were counted. Each well of the 96-well plates was filled with 150 µL of the cell suspension (100,000 cells/mL). The seeded cells were incubated at 37 °C and 5% CO_2 for 24 hours.

2.2.3 Treatment of the cells

Primary dissociated mesencephalic cells on the 12th DIV and neuroblastoma cells 24 h after seeding where concomitantly treated with five different concentrations of CBD (Weltapotheke, Austria) (10, 1, 0.1, 0.01, 0 µM) or THC (Weltapotheke, Austria) (10, 1, 0.1, 0.01, 0 µM) with or without 2-desoxyglucose (10 mM). Every concentration was performed as duplicates.
As the medium from every well was taken out, 340 µL/well on a 48 well-plate and 150 µL/well on a 96-well plate was used as working volume. THC stock (25 mM) and CBD stock (25 mM) are both dissolved in dimethyl sulfoxide (DMSO). Due to this, all wells contain equal amounts (0.08%) of DMSO. Cells were incubated at 37 °C and 5% CO_2 for 48 hours.

2.2.4 Measurements of Cell Parameters

Immunocytochemistry – Anti- tyrosine- hydroxylase staining

Tyrosine-hydroxylase (TH) is an enzyme involved in the synthesis of catecholamine such as dopamine. Dopaminergic neurons can be identified by the anti-tyrosine-hydroxylase positive reaction.

On the 14th day in vitro, cells were fixed using 200 µL/well Accustain^{TM}. After an incubation time of 15 minutes at room temperature (RT), the cells were permeabilized with 200 µL of Triton-X solution (0,4%, dissolved in DPBS) for 30 minutes at RT. Between the following steps, three washing steps were performed using DPBS. To block the unspecific binding sites, horse serum diluted to a 5% solution in DPBS was applied. After the cells were incubated for 90 minutes at RT, anti-tyrosine-hydroxylase mouse antibody (clone 2/40/15, Millipore, USA) diluted in 5% horse serum solution (1:1000) was applied to the cells. The cells were incubated overnight at 4 °C.

On the following day, the Vectastain ABC kit (Vector laboratories, USA) was used. In detail, the secondary biotinylated horse anti mouse antibody (1:200 in DPBS) was applied. During the incubation time of 90 minutes at RT, the avidin/biotin mixture (1:1 in DPBS 1:500) was prepared and let stand for a minimum of 30 minutes to ensure AB complexes were formed. The AB complex solution was applied, and cells incubated for 90 min at RT. Meanwhile a staining solution of 3,3´-diaminobenzidine (DAB) and hydrogen peroxide 30% (H_2O_2) was prepared. For this, 10 mg DAB was dissolved in 10 mL DPBS and separately 0.5 mL DPBS and 20 µL H_2O_2 were combined in an Eppendorf tube. Shortly before application to the cells, the DAB solution and 100 µl of the H_2O_2 solution were combined. DAB/ H_2O_2-solution was applied, and the peroxidase reaction was controlled under an inverted microscope. To preserve the staining, the cells were mounted with Kaiser´s glycerine gelatine. The number of TH-positive cells (THir) and consequently dopaminergic neurons was determined by counting the stained cells using an inverted microscope.

Resazurin reduction assay

To determine the cell viability, the resazurin reduction assay was used in both cell culture systems. As a blue, non-toxic, non-fluorescent, cell permeable substance, resazurin will be reduced to the pink, fluorescent resorufin by NADH within viable mitochondria in the cell, which

can be detected by a plate reader. Resazurin reduction assay can be used as a validated method to generate knowledge about the cellular overall metabolism.
After 48 hours of treatment, to each well of the 96-well plate 15 µL of resazurin solution (500 µM) was added. The content of resorufin was measured at timepoint 0 (t_0) right after the resazurin solution was pipetted into the wells and one hour later (t_1). Cells were incubated at 37°C and 5 % CO_2 between the two measurements. Fluorescent measurement was performed at 570 nm (excitation) and 600nm (emission). The measured data from t_0 were subtracted from t_1 to determine the increase in resorufin content relative to the control.

BCA-Protein assay

Both cell culture systems have been used for the Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA). The measurement of the protein content provides information about the total cell amount after treatment.
This assay is performed immediately after the second measurement of the resazurin reduction assay. The supernatant of each well was removed and 100 µL of RIPA buffer (1:10 diluted with bidest. H_2O, Millipore, USA) was pipetted in each well. The cells were lysed for 10 minutes at 4°C. During this time, the working reagent (18 mL reagent A + 360 µL reagent B) and the standards of bovine serum albumin (BSA, stock solution 2,000 µg/mL) were prepared as follows:

Table 3: standards for BCA Protein assay

BSA concentration [µg/mL]	BSA [µL]	DPBS [µL]
500	50 [stock solution]	150
125	50 [500 µg/mL]	150
50	100 [125 µg/mL]	150
25	100 [50 µg/mL]	100
0		100

After the incubation time of 10 minutes, 25 µL of each sample and each standard was pipetted into a new transparent 96-well plate and 200 µL working reagent was added to each well. Afterwards the plate was incubated at 37 °C and 5 % CO_2 for 30 minutes. Measurement was performed at the absorption maximum of the BCA (562 nm).

ATP assay

Both cell culture systems have been used for this assay. Determination of ATP serves as an indicator of the metabolic status of the cell culture. The CellTiter-Glo® Luminescent Cell Viability Assay (Promega, USA) was used.
For determining the ATP, concentration standards were prepared as follows:

Table 4: standards for ATP assay

100 µM ATP [µL]	DPBS [µL]	Concentration [µM]
100	900	10
50	950	5
10	990	1
0	1000	0

125 µL of each standard was pipetted into a new Eppendorf tube, and 250 µL of working reagent was added. Subsequently, the treatment medium of each well of the white 96-well plate was replaced by 200 µL of the working reagent. The plate and the standards were wrapped up in tin foil and placed on an orbital-shaker at 200 rpm for 30 minutes. After incubation standards were pipetted to the free wells of the sample plate, measurement was performed with a plate reader in the luminescence mode.

2.2.5 Statistics

Statistical evaluation was performed with the Kruskal-Wallis (H)-test followed by a χ^2 test displayed with [*]. To compare the effect of 2-desoxyglucose with the control the Mann-Whitney U-test was performed and displayed with [#]. Both statistical calculations considered $p < 0.05$ as statistically significant.

3 Results

Data obtained in neuroblastoma cells neglect possible cell division of the neuroblastoma cells during the treatment period. Since major differences between the cells under different conditions were not expected, data was not related to the protein content.

3.1 Immunocytochemistry – Anti- tyrosine- hydroxylase staining

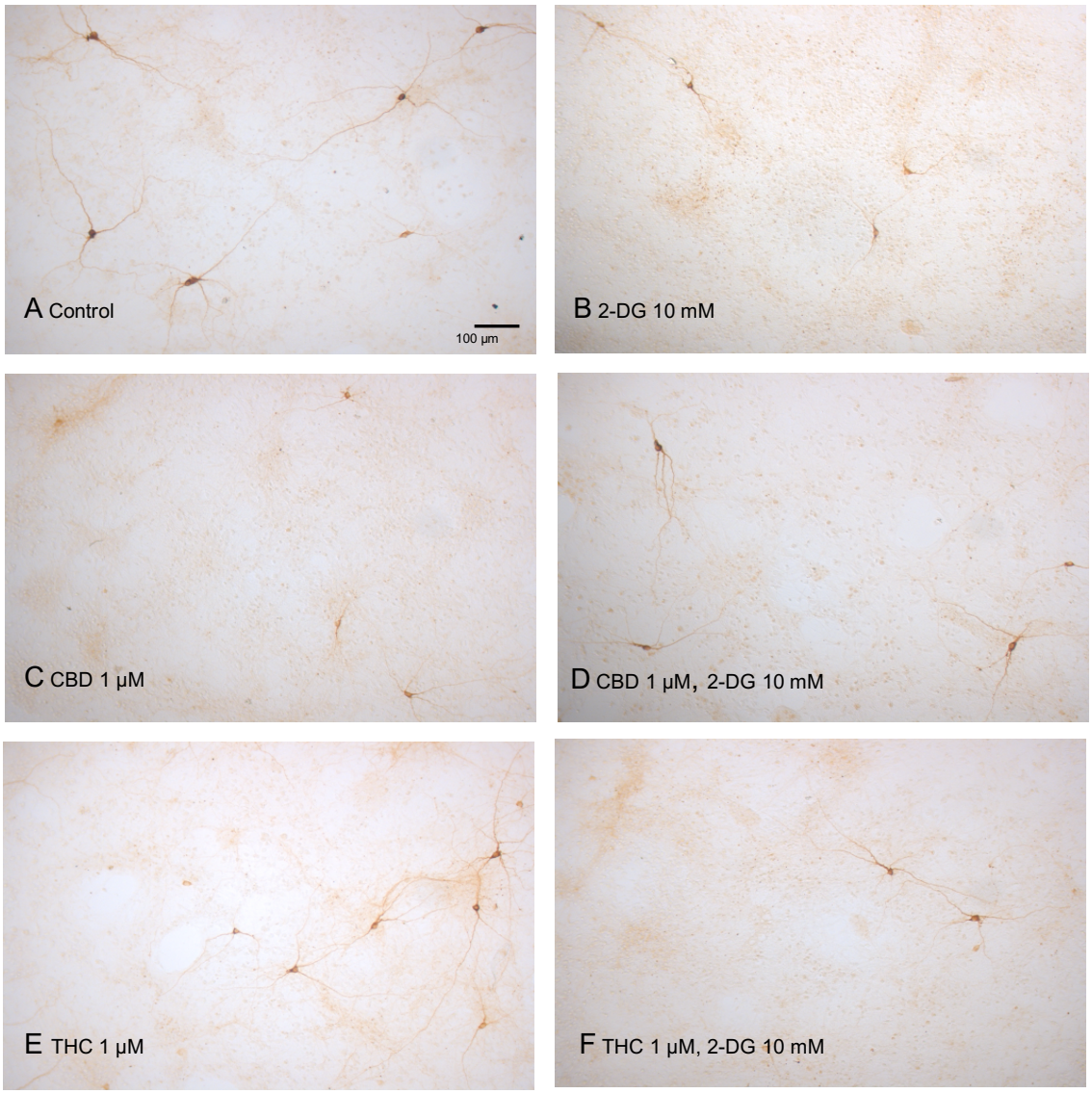

Figure 6: Representative photographs of THir (dopaminergic) neurons after treatment with THC and CBD [1 µM] ± 2-DG [10 mM]. Neurite length and morphology was evaluated optically. (A) Untreated vehicle control shows a high number of dopaminergic neurons with physiologically shaped cell bodies and numerous long neurites. (B) After Treatment with 2-DG, there are only a few dopaminergic neurons with barely pronounced neurites. (C) Treatment with CBD revealed a reduced number of dopaminergic neurons with only a slight number of neurites. (D) CBD co-treated with 2-DG revealed a greater number of dopaminergic neurons and neurites than (C). (E) THC is comparable to the untreated control. (F) THC co-treated with 2-DG showed a lower number of dopaminergic neurons as (E), some neurons displayed almost with strongly degenerated neurites, and some were morphologically well intact neurons.

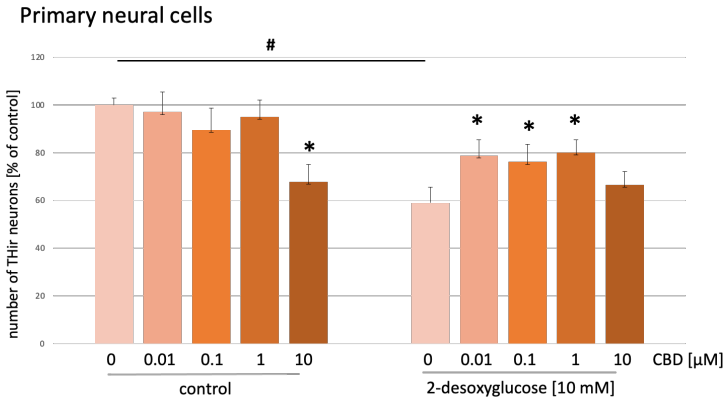

Figure 7: Number THir neurons (dopaminergic neurons) after treatment with CBD alone or co-treated with 2-DG. Data are expressed as means ± SEM of six independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of murine primary mesencephalic cells with 2-DG significantly reduced the number of dopaminergic neurons by 41.0 %, pointing to the inhibition of glycolysis by 2-DG (Fig. 7, Fig. 8).

The effect of CBD: Statistical testing showed in untreated cells a significantly decreased number of dopaminergic neurons by 32.0 % in cultures treated with 10 µM CBD.

In cells treated with 2-DG, no difference was observed at the 10 µM CBD level. In this variant, however, low concentrations of CBD showed a significant increase by 19.8 % (0.01 µM) 17.2 % (0.1 µM) and 21.1 % (1 µM) of the number of dopaminergic neurons related to the effect of 2-DG alone (Fig. 7).

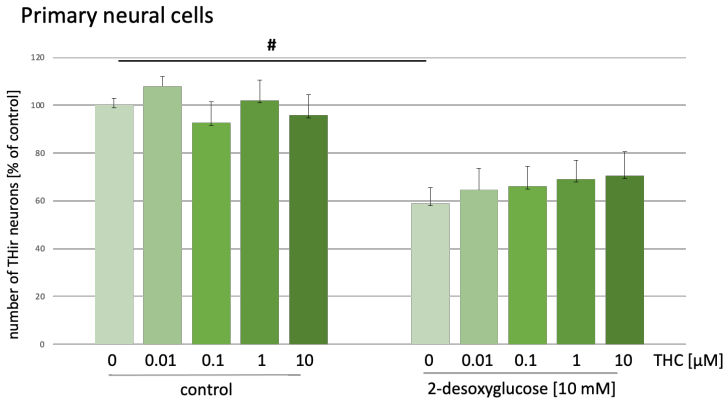

Figure 8: Number THir neurons (dopaminergic neurons) after treatment with THC alone or co-treated with 2-DG. Data are expressed as means ± SEM of six independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: THC had no significant effect on the numbers of dopaminergic neurons within control or 2-DG treatment. In 2-DG treatment, however, a slight trend towards a higher number of dopaminergic neurons with increased THC concentrations could be seen (Fig. 8).

3.2 BCA-Protein assay

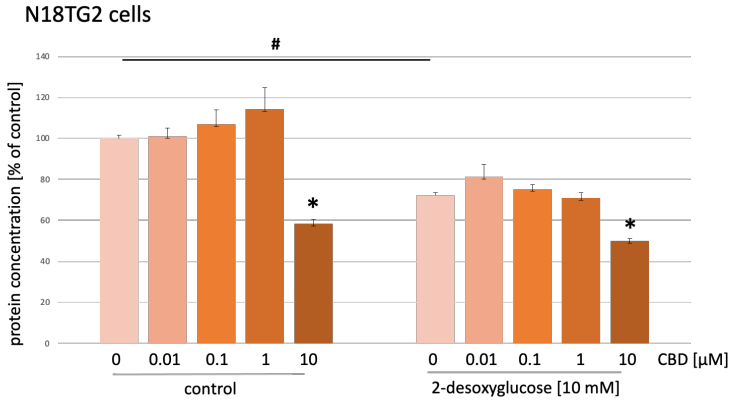

Figure 9: Protein concentration in neuroblastoma cells after treatment with CBD alone or co-treated with 2-DG). A protein concentration of 100% represents the protein content (cell number) in vehicle control cultures. Data are expressed as means ± SEM of nine independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of the N18TG2 neuroblastoma cells with 2-DG significantly reduced the protein content by 27.7 % (Fig. 9, Fig. 10).

The effect of CBD: Kruskal-Wallis (H)-test followed by a χ^2 test revealed a significant toxicity of 10 µM CBD in N18TG2 neuroblastoma cells and therefore a loss of protein by 41.7 %. A trend of increased cell number can be seen at a concentration of 0.01, 0.1, and 1 µM CBD. Low concentrations of CBD, although not significant, showed a modest increase in neuroblastoma cell count, related to the effect of 2-DG alone, namely of 9.0 % (0.01 µM) and 3.0 % (0.1 µM). A significant toxicity of 10 µM CBD co-treated with 2-DG in N18TG2 neuroblastoma cells could be demonstrated by a protein loss of 22.3 % (Fig. 9).

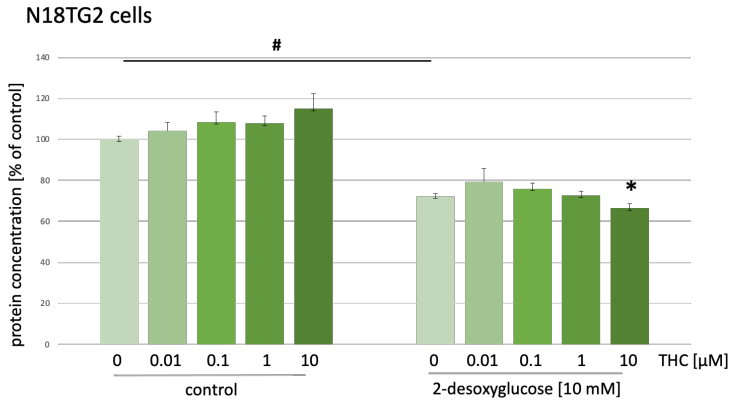

Figure 10: Protein concentration in neuroblastoma cells after treatment with THC alone or co-treated with 2-DG. An absorbance of 100% represents the protein content (cell number) in vehicle control cultures. Data are expressed as means ± SEM of nine independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: Statistical testing showed a significant effect of 10 µM THC co-treated with 2-DG in N18TG2 neuroblastoma cells by a reduction of cell content of 5.8 % compared to the 2-DG control (Fig. 10).

Exposure to low concentrations of THC, although not significant, showed a low protective effect related to the effect of 2-DG of 7.1 % (0.01 µM), 3.7 % (0.1 µM) and 0.3 % (1 µM). A trend of increase in protein levels can be seen at all tested concentrations of THC (Fig. 10).

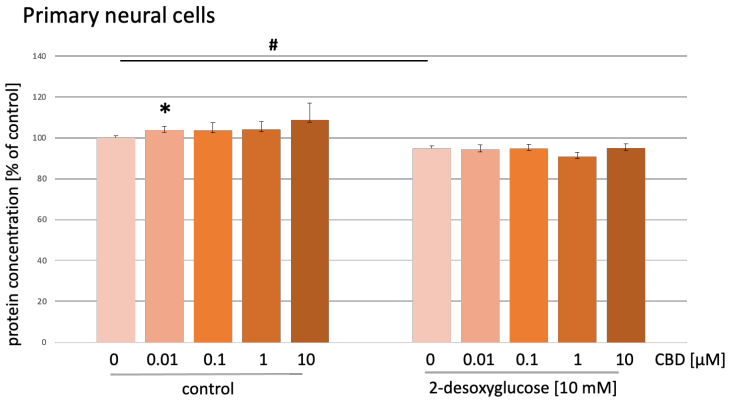

Figure 11: Protein concentration in murine primary mesencephalic cells after treatment with CBD alone or co-treated with 2-DG. A protein concentration of 100% represents the protein levels of untreated vehicle controls. Data are expressed as means ± SEM of five independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of murine primary mesencephalic cells with 2-DG significantly reduced the cell content by 5.2 % (Fig. 11, Fig. 12).

The effect of CBD: A significant increase in protein concentration after treatment with 0.01 µM CBD of 3.7 % could be measured. The protein concentration remained on a similar level during treatment with different concentrations of CBD co-treated with 2-DG (Fig. 11).

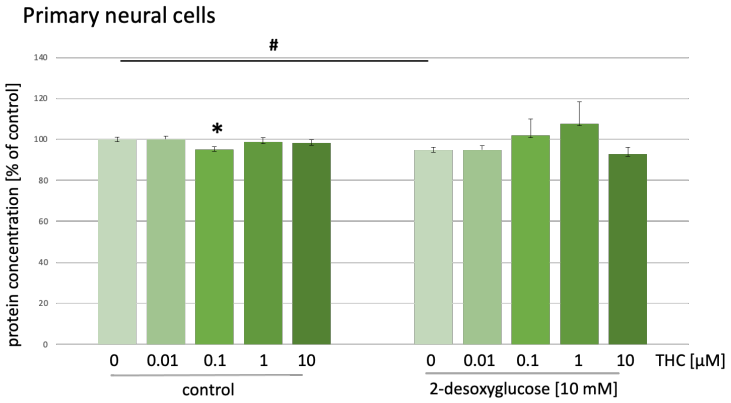

Figure 62: Protein concentration in murine primary mesencephalic cells after treatment with THC alone or co-treated with 2-DG. A protein concentration of 100% represents the protein concentrations in untreated vehicle. controls Data are expressed as means ± SEM of five independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: During THC treatment, the protein concentration remained constant, except a significant loss of protein at 0.1 µM THC by 4.9 %. A clear trend is displayed, whereas at 0.01, 0.1, and 1 µM THC, co-treatment with 2-DG increases the protein content (Fig. 12).

3.3 ATP assay

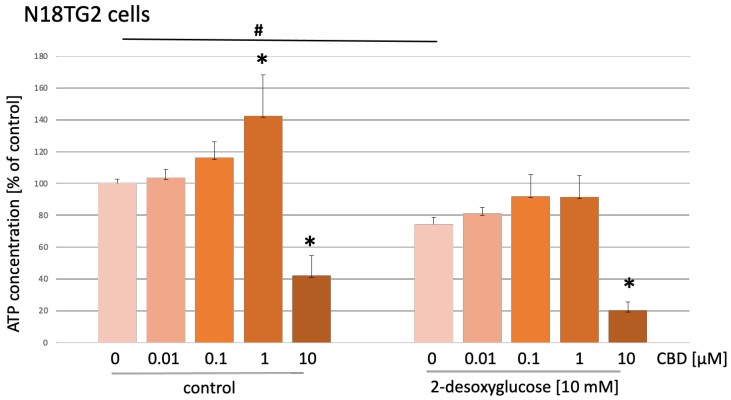

Figure 73: ATP concentration in neuroblastoma cells after treatment with CBD alone or co-treated with 2-DG. An ATP concentration of 100% represents the luminescence emission of untreated vehicle cultures. Data are expressed as means ± SEM of nine independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of the N18TG2 neuroblastoma cells with 2-DG significantly reduced the ATP content by 25.7 % (Fig. 13, Fig. 14).

The effect of CBD: 10 µM of CBD in N18TG2 neuroblastoma significantly reduced ATP concentration by 58.0 %. In contrast, 1 µM CBD showed a significant ATP increase of 42.6 %. A trend of higher ATP concentrations can be detected at a concentration of 0.01 and 0.1 µM CBD as well (Fig. 13).

Low concentrations of CBD co-treated with 2-DG, although not significant, showed a trend of increased ATP concentrations. Contrarily, a significant toxicity of 10 µM CBD co-treated with 2-DG could be demonstrated by a reduction of ATP concentration by 54.1 % compared to 2-DG control (Fig. 13).

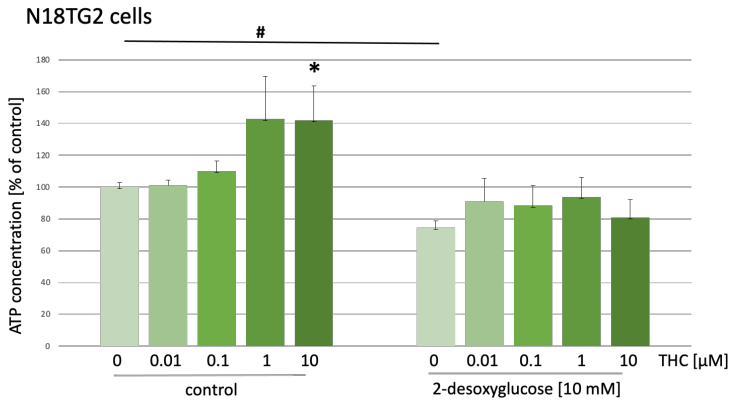

Figure 14: ATP concentration in neuroblastoma cells after treatment with THC alone or co-treated with 2-DG. An ATP concentration of 100% represents the luminescence emission of untreated vehicle cultures. Data are expressed as means ± SEM of nine independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: A significant increase of ATP levels at 10 µM THC by 42.1 % could be observed. A trend of increased ATP concentrations can be seen at a concentration of 0.01, 0.1, and 1 µM CBD as well. Although not significant, THC showed a low protective effect related to the effect of 2-DG in all concentrations used (Fig. 14).

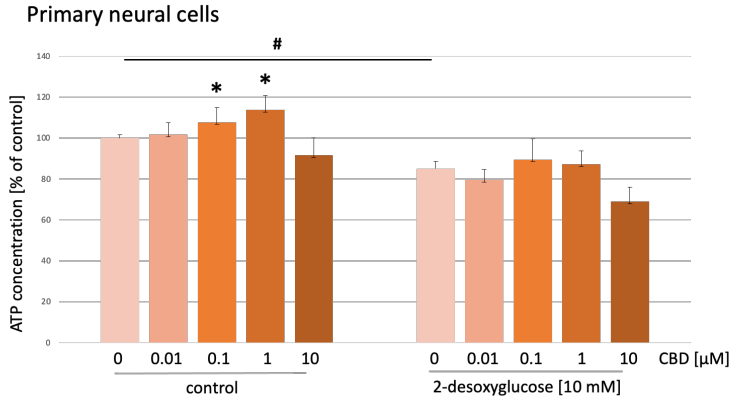

Figure 85: ATP concentration in murine primary mesencephalic cells after treatment with CBD alone or co-treated with 2-DG. An ATP concentration of 100% represents the luminescence emission of untreated vehicle cultures. Data are expressed as means ± SEM of six independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of murine primary mesencephalic cells with 2-DG significantly reduced the ATP concentration by 15.0 % (Fig. 15, Fig. 16).

The effect of CBD: ATP levels increased significantly at 0.1 and 1 µM CBD. Treatment of CBD co-treated with 2-DG revealed no significant differences in all concentrations (Fig. 15).

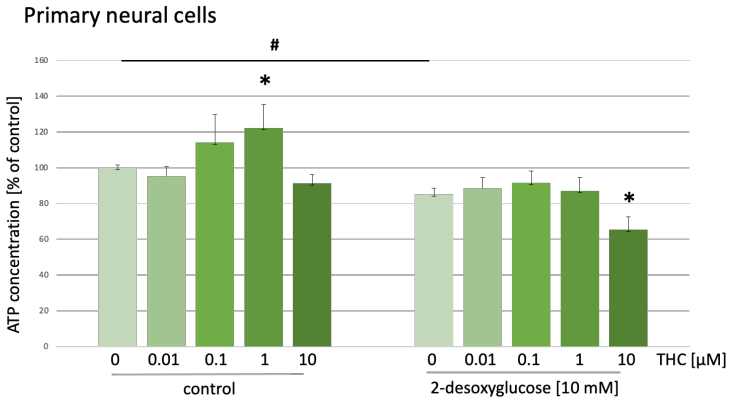

Figure 96: ATP concentration in murine primary mesencephalic cells after treatment with THC alone or co-treated with 2-DG. An ATP concentration of 100% represents the luminescence emission of untreated vehicle cultures. Data are expressed as means ± SEM of six independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: 1 µM of THC, an increase by 22.3 % was detectable. Treatment with 10 µM THC co-treated with 2-DG revealed a significant loss of ATP by 19.7 % compared to the 2-DG control (Fig. 16).

3.4 Resazurin reduction assay

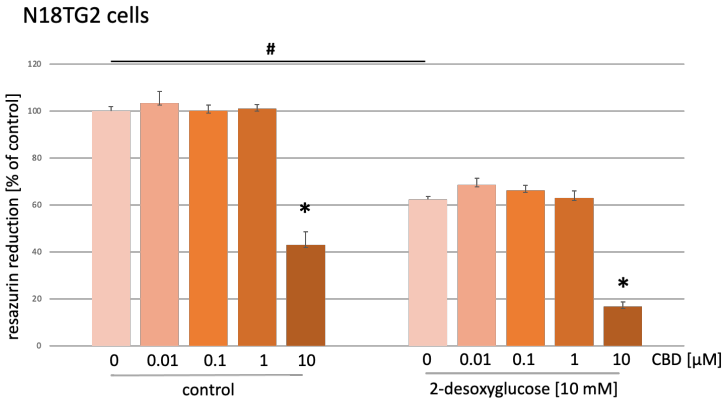

Figure 17: Resazurin reduction in neuroblastoma cells after treatment with CBD alone or co-treated with 2-DG. An absorbance of 100% represents the luminescence emission of untreated DMSO controls. Data are expressed as means ± SEM of nine independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of the N18TG2 neuroblastoma cells with 2-DG significantly reduced the overall cellular metabolism significantly by 37.7 % (Fig. 17, Fig. 18).

The effect of CBD: A significant toxicity of 10 µM CBD was observed in N18TG2 neuroblastoma cells, leading to a reduction of 57.1 % in cellular metabolism. Related to this result, 10 µM CBD co-treated with 2-DG showed a significant diminished overall cellular metabolism by 45.5 % compared to the 2-DG control (Fig. 17).

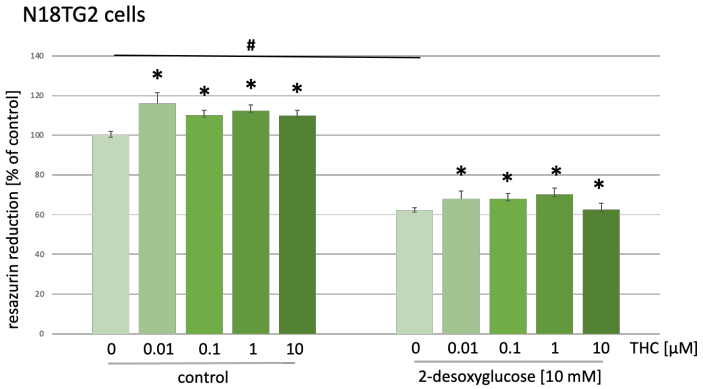

Figure 18: Resazurin reduction in neuroblastoma cells after treatment with THC alone or co-treated with 2-DG. An absorbance of 100% represents the luminescence emission of untreated DMSO controls. Data are expressed as means ± SEM of nine independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: All tested concentrations of THC revealed a significant increase in resazurin reduction. N18TG2 neuroblastoma cells, treated with THC and 2-DG, resulted in a significant trend of increased resazurin reduction (Fig. 18).

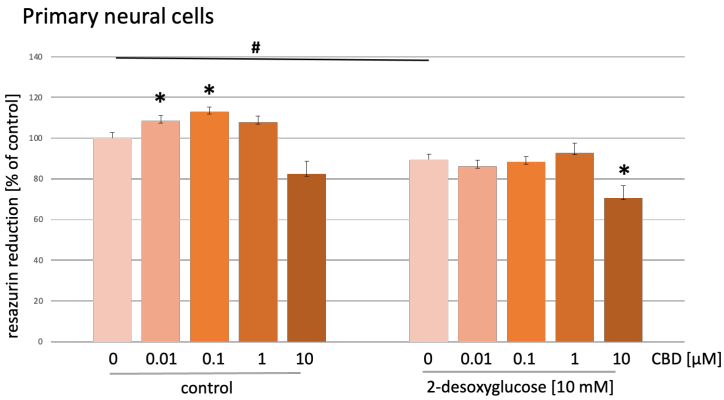

Figure 19: Resazurin reduction in murine primary mesencephalic cells after treatment with CBD alone or co-treated with 2-DG. An absorbance of 100% represents the luminescence emission of untreated DMSO controls. Data are expressed as means ± SEM of five independent experiments. ('*' and '#': $p<0.05$)

The effect of 2-DG: Treatment of murine primary mesencephalic cells with 2-DG significantly reduced the overall cellular metabolism significantly by 10.6 % (Fig. 19, Fig. 20).
The effect of CBD: A significant increased resorufin formation at concentrations of 0.01 and 0.1 µM CBD points to an enhanced overall cellular metabolism. 10 µM of CBD co-treated with 2-DG revealed a significant diminished overall cellular metabolism by 18.7 % (Fig. 19).

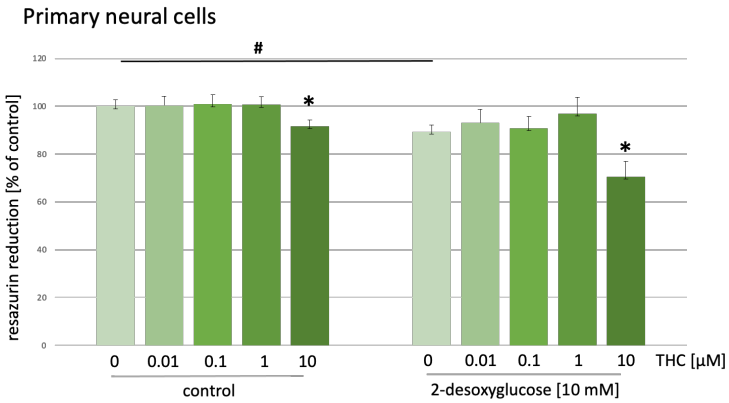

Figure 20: Resazurin reduction in murine primary mesencephalic cells after treatment with THC alone or co-treated with 2-DG. An absorbance of 100% represents the luminescence emission of untreated DMSO controls. Data are expressed as means ± SEM of five independent experiments. ('*' and '#': $p<0.05$)

The effect of THC: A significant decrease of resorufin formation in cultures treated with 10 µM THC alone or together with 2-DG revealed a diminished overall cellular metabolism by 8.3 % (THC, 0) and 18.9 % (THC, 2-DG) (Fig. 20).

4 Discussion

It has to be noted that cell cultures studies shed light on a single time point in a model that not completely reflect the *in vivo* situation. So, the outcome of the study should not be overestimated. In this thesis, the following questions were answered:

(1) Do CBD and THC affect cancer cells to a higher extend than primary neural cell cultures?

CBD in high concentrations is more toxic on cancer cells than on primary neural cell cultures (Figure 9, 13 and 17). This correlates with the present knowledge of neurotoxicity of CBD in high concentrations to N18TG2 cells (41). But primary cell cultures, albeit to a lesser extent, are also affected by the toxicity of CBD (Figure 11, 15 and 19). The Neurochemistry Group of the VetMedUni Vienna demonstrated that CBD at a concentration of 10 µM decreases the viability of dopaminergic neurons in models with induced mitochondiral impairment (8). In our study 10 µM CBD decreased the viabilty of dopaminergic neurons as well (Figure 7). THC does not show any significant differences (Figure 8).

(2) Does the partial inhibition of glycolysis have a greater effect on cancer cells than on primary neural cell cultures?

2-desoyglucose led to a decrease in cell viability in both, neural and cancer cells (all Figures). Based on the acquired data of this study, it can be asserted that neuroblastoma cells suffer from the inhibition of glycolysis to a higher extent than primary neural cell cultures do (all Figures). It remains unclear if this decrease in cell content and metabolism markers (Figure 14 and 16, Figure 18 and 20) is due to cell death or inhibition of mitosis. Since cancer cells have an elevated metabolism, they are more effected by the inhibition of glycolysis.
Chuang and his colleagues demonstrated that 2-DG causes catabolic restrictions and significant loss of ATP content leading to suppressed cell proliferation and apoptosis in neuroblastoma cells, which correlates with the findings of this study (52).

(3) Are there differences in the effects of CBD and THC under glycolytic shortage?

Both phytocannabinoids co-treated with 2-DG were equally neurotoxic at concentrations of 10 µM in primary neural cell cultures (Figure 11, 15 and 19). Low concentrations of CBD and

THC showed no significant effects (Figure 11, 12, 15, 16, 19, 20). CBD exert a greater neuroprotection on dopaminergic neurons then THC does (Figure 7 and 8). THC and low concentrations of CBD have a minor protective effect in cancer cells. CBD acts cytotoxic in high concentrations, but THC does not (Figure 9, 10, 13, 14, 17 and 18).
This correlates with the knowledge about the anticancerogenic effects of CBD (53) and 2-DG (46). Treatment with both anticancerogenic agents emits an enhanced effect on cancer cells.

(4) Do CBD and THC exert neuroprotective effects on primary neural cell cultures, which have been affected by the partial inhibition of glycolysis?

The pathogenesis of the neurodegenerative Parkinson´s disease is mainly characterized trough oxidative stress and mitochondrial impairment which leads to major energy deficits in neurons (54). As CBD and THC are known for their modulation of the mitochondrial function, the effects of both cannabinoids should be considered under additional restriction of another energy-gaining pathway. Primary neural cell cultures represent an optimal model to test the effects of pathogenicity due to the relatively high content of vulnerable dopaminergic neurons, which degenerate due to the pathogeneses of PD.

Low concentrations of THC showed mild protective effects in primary neural cell cultures (Figure 12, 16 and 20). Controversially, 10 µM THC under partial inhibition of glycolysis lowered the ATP concentrations and cellular metabolism (Figure 16 and 20). In addition, THC shows a tendency towards neuroprotective effects on dopaminergic neurons (Figure 8).

CBD reduces the ATP concentration and overall cellular metabolisms, especially in high concentrations (Figure 15 and 19). Although CBD is cytotoxic in primary neural cell cultures it can protect dopaminergic neurons to a small extent (Figure 7). This could be due to the heterogeneity of the primary neural cell culture. Dopaminergic neurons are represented by only 0.1% in a primary neural cell culture. Other cell types, like glia cells, which represent 60% of the culture, are differently affected by 2-DG and thus could balance the values of the total protein content (Figure 10 and 11). Therefore, it can be hypothesized that dopaminergic neurons obtain their energy from another ATP source than oxidative phosphorylation or glycolysis, e.g. from intracellular stores.
Glucose is consumed extensively by neurons and glial cells, although they metabolize glucose differently. Neurons rely on oxidative phosphorylation for energy while glial cells, like astrocytes, rely more on glycolysis. Astrocytes enhance glycolysis by an increased uptake rate

of glutamate, which stimulates glucose uptake to increase lactate production, which is further transferred to neurons. This energy-generating mechanism for neurons is called "astrocyte-to-neuron lactate shuffle" (55).
Looking at the protein content change in primary neural cell cultures treated with CBD compared to the treatment in combination with 2-DG, there is no noticeable change in the protein content in co-treated cultures (Figure 11 and 12). In this case, it can be assumed that CBD puts the cells into quiescent state, rather than leading to cellular death even both energy-generating sources of the cells are partially inhibited.

(5) Are neurotoxic effects of CBD more accentuated in cancer cell than in primary neural cell cultures when glycolysis is partially inhibited?

CBD is discussed for its anticancerogenic properties. Hamad and Olsen (56) demonstrated CBD´s anticancerogenic properties in lung cancer cells. They found CBD to decrease the viability and induce cell death in this cancer cells. CBD also increased the expression of pro-apoptotic proteins, increased the ROS levels, decreased the self-renewal, and impaired the mitochondrial activity. Mitochondrial impairment, as one reason of CBDs anticancerogenic properties was also displayed by (41). According to this study, CBD reduced the mitochondrial respiration on complex I and II (41).
Cancer cells are known for their metabolic conversion. Some cancer cells change their pathway to obtain energy for continues proliferation and migration from oxidative phosphorylation to aerobic or anaerobic glycolysis (45). Due to the additional inhibition of glycolysis with 2-DG, we expect reduced mitosis rates or an increase in cell death in neuroblastoma cells.
CBD (10 µM) act cytotoxic in both, primary neural cells, and cancer cells co-treated with 2-DG, whereby cancer cells are affected by a higher toxicity. Resazurin reduction, protein concentration and ATP concentration decrease significantly compared to lower concentrations of CBD (Figure 9, 11, 13, 15, 17 and 19).

5 Conclusion

In recent years, phytocannabinoids such as cannabidiol (CBD) and tetrahydrocannabinol (THC) have been studied for potential medical applications. However, there is still much to learn about the effects of these compounds. The effects of CBD and THC on control as well as cancer cells with or without reduced glycolysis activity were examined in this study.

The hypothesis, that in cells with reduced glycolysis activity, treatment with CBD, but not THC, leads to cell degenerative effects could be confirmed in the N18TG2 neuroblastoma cells, but not in primary neural cell cultures.
N18TG2 neuroblastoma cells, the model for cancer cells in our study, showed clear degenerative effects when applying CBD, but not by THC.
In primary neural cell cultures, THC and CBD affected the cells positively to a similar extent, except for high concentrations, which were neurotoxic. CBD positively influences dopaminergic neurons suffering from glycolytic shortage.
Without overinterpretation I conclude that cell culture data did not point to a risk when low glucose intake meets phytocannabinoid treatment in strategies against cancer or neurodegenerative diseases. Nonetheless, further investigations in *in vivo* models are necessary to test our *in vitro* results.

6 References

1. Corroon, J.; Felice, JF. (2019) The Endocannabinoid System and its Modulation by Cannabidiol (CBD). Alternative Therapies. 25(2).
2. Lambert, DM. (2009). Cannabinoids in Nature and Medicine. Weinheim, Cambridge: Whiley-VCH.
3. di Marzo, V.; Piscitelli, F. (2015). The Endocannabinoid System and its Modulation by Phytocannabinoids. Neurotherapeutics. Springer New York LLC. 12: 692–8.
4. Navarrete, F.; García-Gutiérrez, MS.; Jurado-Barba, R.; Rubio, G.; Gasparyan, A.; Austrich-Olivares, A. et al. (2020). Endocannabinoid System Components as Potential Biomarkers in Psychiatry. Frontiers in Psychiatry. 11:315.
5. Batinga, H. (2016). Cannabinoid receptor ligands prevent dopaminergic neurons death induced by neurotoxic, inflammatory, and oxidative stimuli in vitro. Journal of Biomedical Sciences. 05(01):2.
6. Baker, D.; Pryce, G.; Giovannoni, G.; Thompson, AJ. (2003). The therapeutic potential of cannabis. Lancet Neurology. 2(5):291–8.
7. Pertwee, RG. (1997). Pharmacology of Cannabinoid CB1 and CB2 Receptors. 74(2):129-180.
8. Moldzio, R.; Pacher, T.; Krewenka, C.; Kranner, B.; Novak, J.; Duvigneau, JC. et al. (2012) Effects of cannabinoids Δ(9)-tetrahydrocannabinol, Δ(9)-tetrahydrocannabinolic acid and cannabidiol in MPP+ affected murine mesencephalic cultures. Phytomedicine. 19(8–9):819–24.
9. Campos, AC.; Fogaça, MV.; Sonego, AB.; Guimarães, FS. (2016). Cannabidiol, neuroprotection and neuropsychiatric disorders. Pharmacological Research. 112:119–27.
10. Deutsch, DG. (2016). A personal retrospective: Elevating anandamide (AEA) by targeting fatty acid amide hydrolase (FAAH) and the fatty acid binding proteins (FABPs). Front Pharmacol. 7:370.
11. Robbe, D.; Kopf, M.; Remaury, A.; Bockaert, J.; Manzoni, OJ. (2002). Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. www.pnas.orgcgidoi10.1073pnas.122149199 (access 05.10.2022).
12. Andre, CM.; Hausman, JF.; Guerriero, G. (2016) Cannabis sativa: The plant of the thousand and one molecules. Front. Plant Sci. 7:19.
13. Hampson, AJ.; Grimaldi, M.; Axelrod, J.; Wink, D. (1998). Cannabidiol and Δ9-tetrahydrocannabinol are neuroprotective antioxidants. Medical Sciences. 95:8268-8273.
14. Britch, SC.; Babalonis, S.; Walsh, SL. (2021) Cannabidiol: pharmacology and therapeutic targets. Psychopharmacology. 238:9-28.

15. Poff, A.; Koutnik, AP.; Egan, KM.; Sahebjam, S.; D'Agostino, D.; Kumar, NB. (2019). Targeting the Warburg effect for cancer treatment: Ketogenic diets for management of glioma. Semin Cancer Biol. 56:135–48.
16. Cifelli, P.; Ruffolo, G.; de Felice, E.; Alfano, V.; van Vliet, EA.; Aronica, E. et al. (2020). Phytocannabinoids in neurological diseases: Could they restore a physiological gabaergic transmission? International Journal of Molecular Sciences. 21(3):723.
17. Maroon, J.; Bost, J. (2018). Review of the neurological benefits of phytocannabinoids. Surgical Neurology International. Scientific Scholar.9(1):91.
18. Pertwee, RG. (2008). The diverse CB 1 and CB 2 receptor pharmacology of three plant cannabinoids: Δ 9-tetrahydrocannabinol, cannabidiol and Δ 9-tetrahydrocannabivarin. British Journal of Pharmacology.153:199–215.
19. Reith, W. (2018). Neurodegenerative Erkrankungen. Radiologe.58(3):241–58.
20. Lastres-Becker, I.; Molina-Holgado, F.; Ramos, JA.; Mechoulam, R.; Fernández-Ruiz, J. (2005). Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: Relevance to Parkinson's disease. Neurobiol Dis. 19(1–2):96–107.
21. Moujalled, D.; Strasser, A.; Liddell, JR. (2021). Molecular mechanisms of cell death in neurological diseases. Cell Death and Differentiation. Springer Nature. 28:2029–44.
22. Schieber, M.; Chandel, NS. (2014). ROS function in redox signaling and oxidative stress. Current Biology. 24(10):453-462.
23. Illarioshkin, SN.; Klyushnikov, SA.; Vigont, VA.; Seliverstov, YA.; Kaznacheyeva, EV. (2018). Molecular Pathogenesis in Huntington's Disease. Biochemistry (Moscow). Pleiades journals. 83:1030–9.
24. Lin, F.; Luo, SQ. (2019). Mitochondria in neurodegenerative diseases. CNS Neuroscience and Therapeutics. Blackwell Publishing Ltd. 25:813–5.
25. Breijyeh, Z.; Karaman, R. (2020). Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Molecules. MPDI. 25(24):5789.
26. Šerý, O.; Povová, J.; Míšek, I.; Pešák, L.; Janout, V. (2013). Molecular mechanisms of neuropathological changes in Alzheimer's disease: A review. Folia Neuropathologica. Termedia Publishing House Ltd. 51:1–9.
27. Abate, G.; Uberti, D.; Tambaro, S. (2021). Potential and limits of cannabinoids in alzheimer's disease therapy. Biology (Basel). 10(6):542.
28. Currais, A.; Quehenberger, O.; Armando, AM.; Daugherty, D.; Maher, P.; Schubert, D. (2016). Amyloid proteotoxicity initiates an inflammatory response blocked by cannabinoids. NPJ Aging Mech Dis. 2(1).
29. Marsicano, G.; Lafenêtre, P. (2009). Roles of the Endocannabinoid System in Learning and Memory. In: Kendall, D.; Alexander, S. (eds). Behavioral Neurobiology of the Endocannabinoid System. Current Topics in Behavioral Neurosciences. Springer, Berlin, Heidelberg. 1:201-230.

30. Esposito, G.; de Filippis, D.; Steardo, L.; Scuderi, C.; Savani, C.; Cuomo, V. et al. (2006). CB1 receptor selective activation inhibits β-amyloid-induced iNOS protein expression in C6 cells and subsequently blunts tau protein hyperphosphorylation in co-cultured neurons. Neuroscience Letter. 404(3):342–6.
31. Libro, R.; Diomede, F.; Scionti, D.; Piattelli, A.; Grassi, G.; Pollastro, F. et al. (2017). Cannabidiol modulates the expression of alzheimer's disease-related genes in mesenchymal stem cells. International Journal Molecular Science. 18(1).
32. Blázquez, C.; Chiarlone, A.; Sagredo, O.; Aguado, T.; Pazos, MR.; Resel, E. et al. (2011). Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. Brain. 134(1):119–36.
33. Sagredo, O.; Ramos, JA.; Decio, A.; Mechoulam, R.; Fernández-Ruiz, J. (2007). Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. European Journal of Neuroscience. 26(4):843–51.
34. Aymerich, MS.; Aso, E.; Abellanas, MA.; Tolon, RM.; Ramos, JA.; Ferrer, I. et al. (2018). Cannabinoid pharmacology/therapeutics in chronic degenerative disorders affecting the central nervous system. Biochemical Pharmacology. 157:67–84.
35. Masrori, P.; van Damme, P. (2020). Amyotrophic lateral sclerosis: a clinical review. European Journal of Neurology. Blackwell Publishing Ltd. 27:1918–29.
36. Fernandez-Ruiz, J.; Espejo-Porras, F.; García-Toscano, L.; Rodríguez-Cueto, C.; Santos-García, I.; de Lago, E. (2019). Targeting glial cannabinoid CB 2 receptors to delay the progression of the pathological phenotype in TDP-43 (A315T) transgenic mice, a model of amyotrophic lateral sclerosis. Cannabinoid Research British Journal of Pharmacology British Journal of Pharmacology. 176:1585-1600.
37. Olanow, CW. (2007). The pathogenesis of cell death in Parkinson's disease. Movement Disorders. 22(17):335-342.
38. Gardoni, F.; di Luca, M. (2006). New targets for pharmacological intervention in the glutamatergic synapse. European Journal of Pharmacology. 545:2–10.
39. Murillo-Rodríguez, E.; Palomero-Rivero, M.; Millán-Aldaco, D.; Mechoulam, R.; Drucker-Colín, R. (2011). Effects on sleep and dopamine levels of microdialysis perfusion of cannabidiol into the lateral hypothalamus of rats. Life Science. 88(11–12):504–11.
40. Norris, C.; Loureiro, M.; Kramar, C.; Zunder, J.; Renard, J.; Rushlow, W. et al. (2016). Cannabidiol modulates fear memory formation through interactions with serotonergic transmission in the mesolimbic system. Neuropsychopharmacology. 41(12):2839–50.
41. Unterberger, A. (2016). Neuroprotective Effects of Phytocannabinoids: Effects of THC against radical induced oxidative stress and influences of CBD and THC on mitochondrial function Master Thesis. Vienna. Medical Biochemistry.

42. Rigoulet, M.; Bouchez, CL.; Paumard, P.; Ransac, S.; Cuvellier, S.; Duvezin-Caubet, S. et al. (2020). Cell energy metabolism: An update. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1861(11):148276.
43. https://www.ck12.org/biology/krebs-cycle/lesson/Krebs-Cycle-BIO/ (access 18.12.22, 22:40).
44. Liu, KG.; Kim, JI.; Olszewski, K.; Barsotti, AM.; Morris, K.; Lamarque, C. et al. (2020). Discovery and Optimization of Glucose Uptake Inhibitors. J Med Chem. 63(10):5201–11.
45. Zhang, D.; Li, J.; Wang, F.; Hu, J.; Wang, S.; Sun, Y. (2014). 2-Deoxy-D-glucose targeting of glucose metabolism in cancer cells as a potential therapy. Cancer Letters. 355(2):176–83.
46. Pajak, B.; Siwiak, E.; Sołtyka, M.; Priebe, A.; Zieliński, R.; Fokt, I. et al. (2020). 2-Deoxy-D-Glucose and its analogs: From diagnostic to therapeutic agents. International Journal of Molecular Sciences. 21(1):234.
47. Aghaee, F.; Islamian, JP.; Baradaran, B. (2012). Enhanced radiosensitivity and chemosensitivity of breast cancer cells by 2-deoxy-D-glucose in combination therapy. Journal of Breast Cancer. 15:141–7.
48. Gaven, F.; Marin, P.; Claeysen, S. (2014). Primary Culture of Mouse Dopaminergic Neurons. J Vis Exp. e51751(doi:10.3791/51751).
49. Cellosaurus. Cellosaurus cell line N18TG2 (CVCL_2132), https://web.expasy.org/cellosaurus/CVCL_2132, (access on 30/04/2020).
50. Svatunek, S. (2020). Toxicity of Cannabidiol on Brain Cancer Cells and Murine Mesencephalic Primary Cultures under Inhibition of Glycolysis. Bachelor Thesis. Vienna. Medical Biochemistry.
51. Radad, K.; Gille, G.; Moldzio, R.; Saito, H.; Ishige, K.; Rausch, WD. (2004). Ginsenosides Rb1 and Rg1 effects on survival and neurite growth of MPP +-affected mesencephalic dopaminergic cells. Journal Neural Transmission. 111(1):37–45.
52. Chuang, JH.; Chou, MH.; Tai, MH.; Lin, TK.; Liou, CW.; Chen, T. et al. (2013). 2-Deoxyglucose treatment complements the cisplatin- or BH3-only mimetic-induced suppression of neuroblastoma cell growth. International Journal Biochemistry Cell Biology. 45(5):944–51.
53. Seltzer, ES.; Watters, AK.; Mackenzie, D.; Granat, LM.; Zhang, D. (2020). Cannabidiol (CBD) as a promising anti-cancer drug. Cancers. MDPI. 12:1–26.
54. Saxena, U. (2012). Bioenergetics failure in neurodegenerative diseases: Back to the future. Expert Opinion on Therapeutic Targets. 16:351–4.
55. Magistretti, PJ. Allaman, I. (2015). A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. Neuron. Cell Press. 86(4):883–901.
56. Hamad, H.; Olsen, BB. (2021). Cannabidiol induces cell death in human lung cancer cells and cancer stem cells. Pharmaceuticals. 14(11):1169.

7 Appendix

7.1 Table Index

7.2 Figure Index