Charles University

Faculty of Pharmacy in Hradec Králové

Department of Pharmacology and Toxicology

Placental homeostasis of monoamines; effect of gestation age

Diploma thesis

Diploma thesis supervisor: Prof. PharmDr. František Štaud, Ph.D.

Consultant: PharmDr. Rona Karahoda, Ph.D.

Hradec Králové 2022 Filip Mahrla

STATEMENT OF AUTORSHIP

I hereby declare that I am the sole author of this diploma thesis and that I have not used any sources other than those listed in the bibliography and identified as references. I further declare that I have not submitted this thesis at any other institution in order to obtain a degree.

In Hradec Králové Filip Mahrla

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ABSTRACT

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Catecholamines norepinephrine and dopamine have been implicated in numerous physiological processes within the central nervous system. Emerging evidence suggests their involvement in placental development and functions and a crucial role in fetal development and programming. Nonetheless, a comprehensive characterization of catecholamine synthesis, degradation, and transport in the fetoplacental unit is still lacking. Thus, in this thesis, we aimed to provide a comprehensive evaluation of catecholamine metabolism and transport in the fetoplacental unit. Gene and protein expression was evaluated using quantitative polymerase chain reaction (PCR) and Western blot analysis, respectively. Firstly, using several placental cell models (BeWo, JEG-3, primary trophoblast cells), we identified components of cellular catecholamine handling associated with the trophoblast cells. Next, we determined the effect of advancing gestation on the placental catecholamine system in humans (first trimester vs. term placenta) and rats (gestation day 15, 18, and 21). Lastly, we addressed the expression of catecholamine pathway in rat fetal organs (brain, intestine, liver, lungs, kidneys, and heart) from mid-to-late gestation. Collectively, we suggest that during pregnancy, regulatory pathways control levels of norepinephrine and dopamine in the fetoplacental unit to ensure proper embryo and fetal development throughout gestation.

ABSTRAKT

Univerzita Karlova

Farmaceutická fakulta v Hradci Králové

Katedra farmakologie a toxikologie

Student: Filip Mahrla

Školitel: Prof. PharmDr. František Štaud, Ph.D.

Konzultant: PharmDr. Rona Karahoda, Ph.D.

Název diplomové práce: Placentární homeostáza monoaminů v průběhu těhotenství

Katecholaminy noradrenalin a dopamin se podílejí na řadě fyziologických procesů v centrálním nervovém systému. Objevující se důkazy naznačují jejich zapojení do vývoje a funkcí placenty a klíčovou roli ve vývoji a programování plodu. Komplexní charakterizace syntézy, degradace a transportu katecholaminů ve fetoplacentární jednotce však stále chybí. V této diplomové práci jsme se tedy zaměřili na komplexní hodnocení metabolismu a transportu katecholaminů ve fetoplacentární jednotce. Exprese genů a proteinů byla hodnocena pomocí kvantitativní polymerázové řetězové reakce (PCR) a analýzy Western blot. Nejprve jsme pomocí několika modelů placentárních buněk (BeWo, JEG-3, primární trofoblastové buňky) identifikovali komponenty buněčného řízení katecholaminů spojené s buňkami trofoblastu. Dále jsme určili účinek postupující březosti na placentární katecholaminový systém u lidí (první trimestr vs. termální placenta) a potkanů (15., 18. a 21. den březosti). Nakonec jsme se zabývali expresí katecholaminové dráhy ve fetálních orgánech potkana (mozek, střevo, játra, plíce, ledviny a srdce) od poloviny do pozdního těhotenství. Společně usuzujeme, že regulační dráhy během těhotenství kontrolují hladiny noradrenalinu a dopaminu ve fetoplacentární jednotce, aby byl zajištěn správný vývoj embrya a plodu během těhotenství.

Contents

1 LIST OF ABBREVIATIONS

ABC – ATP binding cassette transporter

AD – aldehyde dehydrogenase

AR – aldehyde reductase

B2M – β-2-microglobulin

cDNA – complementary DNA

CNS – central nervous system

COMT – catechol-o-methyl transferase

CTB – cytotrophoblast

DAT/SLC6A3 – dopamine transporter

DBH – dopamine β-hydroxylase

DDC – dopa decarboxylase

DHMA – 3,4-dihydroxymethamphetamine

DHPG – 3,4-dihydroxy phenyl glycol

DMEM – Dulbecco's modified eagle medium

DNA – deoxyribonucleic acid

DOHaD – developmental origins of health and disease

DOPAC – 3,4-dihydroxyphenylacetic acid

DOPAL – 3,4-dihydroxy phenylacetaldehyde

DOPEGAL – 3,4-dihydroxy phenyl glycolaldehyde

DOPET – 3,4-dihydroxy phenyl ethanol

FBS – fetal bovine serum

GADPH – glyceraldehyde 3-phosphate dehydrogenase

GD – gestation day

hCG – human chorionic gonadotropin

HVA – homovanillic acid

MAO – monoamine oxidase

MAO-A – monoamine oxidase type A

MEM – 2-methoxyethoxymethyl ether

MHPG – vanylglycol

mRNA – messenger ribonucleic acid

MRP2 – multidrug resistance-associated protein 2

NET/SLC6A2 – norepinephrine transporter

OCT3/SLC22A3 – organic cation transporter 3

PBS – phosphate-buffered saline

PHT – primary human trophoblast

PNMT – phenylethanolamine-N-methyl transferase

PVDF – polyvinylidene fluoride

qPCR – quantitative polymerase chain reaction

RNA – ribonucleic acid

SERT/SLC6A4 – serotonin transporter

SLC – solute carrier transporter

STB – syncytiotrophoblast

TH – tyrosine hydroxylase

Ywhaz – tyrosine 3-Monooxygenase/tryptophan 5-monooxygenase activation protein zeta

2 INTRODUCTION

The catecholamines dopamine and norepinephrine are important bioactive molecules participating in various functions within the body (Nagatsu 2007). Catecholamine handling in tissues can be separated into synthesis, metabolism, transport, and action on target receptors (Ganapathy, Ramamoorthy et al. 1993). Catecholamines are synthesized from the non-essential amino acid tyrosine. Hydroxylation via tyrosine hydroxylase (TH) yields L-dopa, which is subsequently decarboxylated by dopa decarboxylase (DDC) to yield dopamine (Coyle and Axelrod 1972, Siaterli, Vassilacopoulou et al. 2003). One more hydroxylation step by dopamine β hydroxylase leads to the production of norepinephrine, which can further be methylated by phenylethanolamine N methyl transferase (PNMT) to produce epinephrine (Axelrod 1971). Metabolism of catecholamines is mainly mediated via the action of monoamine oxidase (MAO) and catechol-o-methyltransferase (COMT) (Axelrod 1971). On the other hand, active transport of catecholamines is facilitated chiefly through two transporters, namely the dopamine (DAT/SLC6A3) and norepinephrine (NET/SLC6A2) transporter (Ganapathy, Ramamoorthy et al. 1993).

During the prenatal period, catecholamines are critical for the normal functioning of the placenta as well as fetal development and programming (Rosenfeld 2021). As the interphase between the mother and the fetus, the placenta is an essential organ which plays a critical role in the transport of nutrients, synthesis and metabolism of several hormones, and providing immunological protection (Staud and Karahoda 2018). Although the placenta is a non-neuronal tissue, several lines of evidence have shown that it is equipped with proteins which participate in the synthesis, metabolism, and transport of neuroactive compounds (Ganapathy, Ramamoorthy et al. 1993, Cote, Fligny et al. 2007, Bonnin, Goeden et al. 2011, Karahoda, Horackova et al. 2020). Recently, the placenta has been suggested as a dopaminergic and noradrenergic organ (Rosenfeld 2021). Importantly, the origin of many neurobehavioral disorders is now believed to trace back to pathological alterations within the placenta and its functions. Thus, placental handling of neuroactive compounds, including catecholamines, is increasingly implicated with developmental origins of adult health and disease (DOHaD) (Bonnin and Levitt 2011, Kratimenos and Penn 2019). Nonetheless, we still are limited in the understanding of physiological aspects of catecholamine handling in the placenta. Moreover, as a continuously growing organ, the placenta experiences physiological changes throughout pregnancy to adapt to its own and fetal demands for nutrients (Novotna, Libra et al. 2004, Meyer zu Schwabedissen, Jedlitschky et al. 2005, Lee, Hebert et al. 2013, Karahoda, Abad et al. 2020).

Considering the role of catecholamines for placental and fetal development, it is essential to decipher the gestational-age dependent changes in the expression and function of enzymes and transporters involved in catecholamine synthesis, metabolism, and transport. In addition, it is essential to identify the contribution of fetal organs in catecholamine handling in the fetoplacental unit. Such knowledge may help in identification of mechanisms involved in placental pathologies and fetal programming of mental health disorders.

3 BACKGROUND

3.1 Biosynthesis and metabolism of monoamines

Catecholamines are monoamine neurotransmitters consisting of a catechol moiety. Catecholamine biosynthesis (Figure 1) starts with the hydroxylation of the amino acid tyrosine by TH, resulting in the production of dihydroxyphenylalanine (DOPA) (Coyle and Axelrod 1972). This reaction represents the rate-limiting step in catecholamine synthesis and it requires oxygen as a co-substrate and tetrahydrobiopterin as a cofactor (Coyle and Axelrod 1972). In the next step, DOPA is decarboxylased by DDC, generating dopamine (Siaterli, Vassilacopoulou et al. 2003). Most of dopamine production happens in the brain. Norepinephrine is synthesized from dopamine by dopamine β-hydroxylase (DBH) and in the last step, PNMT leads to the production of epinephrine (Axelrod 1971).

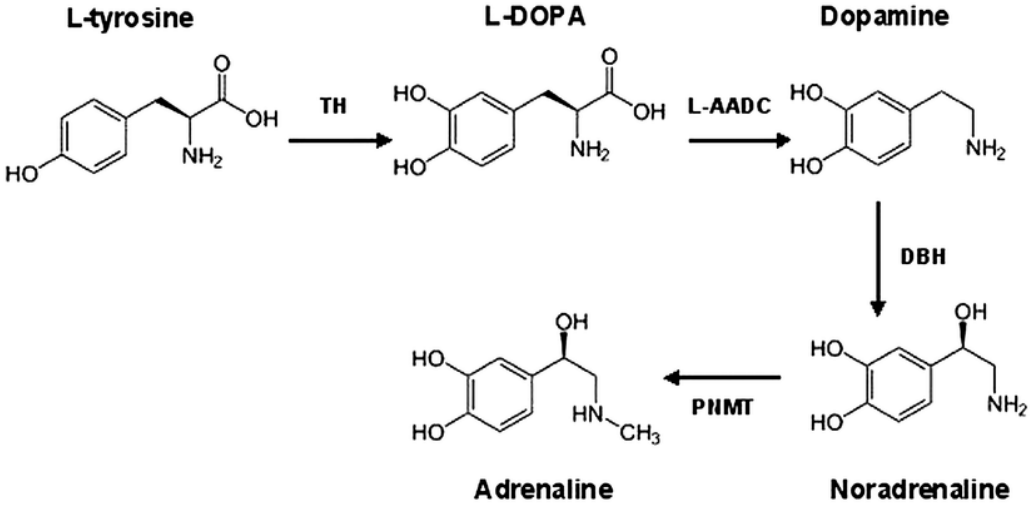

Figure 1. Biosynthesis of monoamines. Adopted from (Ruohonen 2022).

In the brain, newly synthetised catecholamines at the pre-synaptic nerve terminals are mostly released by the mechanism of exocytosis from vesicles into the synapse, where they act as neurotransmitters (Schulz, Eisenhofer et al. 2004). Then they are diffused through the synaptic cleft, which enables them to react with specific monoamines receptors, specifically the dopamine receptors D_1-D_5 and adrenergic receptors α_1, α_2 and β_1, β_2, β_3 (Schulz, Eisenhofer et al. 2004). This reaction leads to the transmission of chemical information.

Catecholamines are degraded mainly via two enzymatic reactions mediated by MAO and COMT (Figure 2) (Schulz, Eisenhofer et al. 2004). The first step in the degradation of catecholamines is deamination, which is catalyzed by MAO and deaminated aldehyde

intermediates are created. Dopamine is changed to 3,4-dihydroxy phenylacetaldehyde (DOPAL), while norepinephrine and epinephrine are both transformed to 3,4-dihydroxy phenyl glycolaldehyde (DOPEGAL). Aldehyde dehydrogenase (AD) and aldehyde reductase (AR) are the key enzymes in the next step of metabolism of monoamines. DOPAL is converted either to 3,4-dihydroxyphenylacetic acid (DOPAC) by AD or to 3,4-dihydroxy phenyl ethanol (DOPET) by AR. Additionally, AD changes DOPEGAL to 3,4-dihydroxymethamphetamine (DHMA) and AR to 3,4-dihydroxy phenyl glycol (DHPG). DOPAL prefers AD, because of the lack of the beta-hydroxyl group, so the formation of DOPAC is higher than formation of DOPET. On the other side, DOPEGAL contains beta-hydroxyl group, so it preferentially binds AR and therefore the creation of DHPG is favoured. The third step is called O-methylation, which requires COMT. In the reaction with DOPAC, the homovanillic acid (HVA) is created which ends the metabolic pathway of dopamine. COMT converts DHPG to vanylglycol (MHPG). In the very last step, the vanillylmandelic acid (VMA) is made from MHPG, which is the end-product of metabolism of norepinephrine and epinephrine. Both HVA and VMA are excreted in urine (Paravati, Rosani et al. 2022).

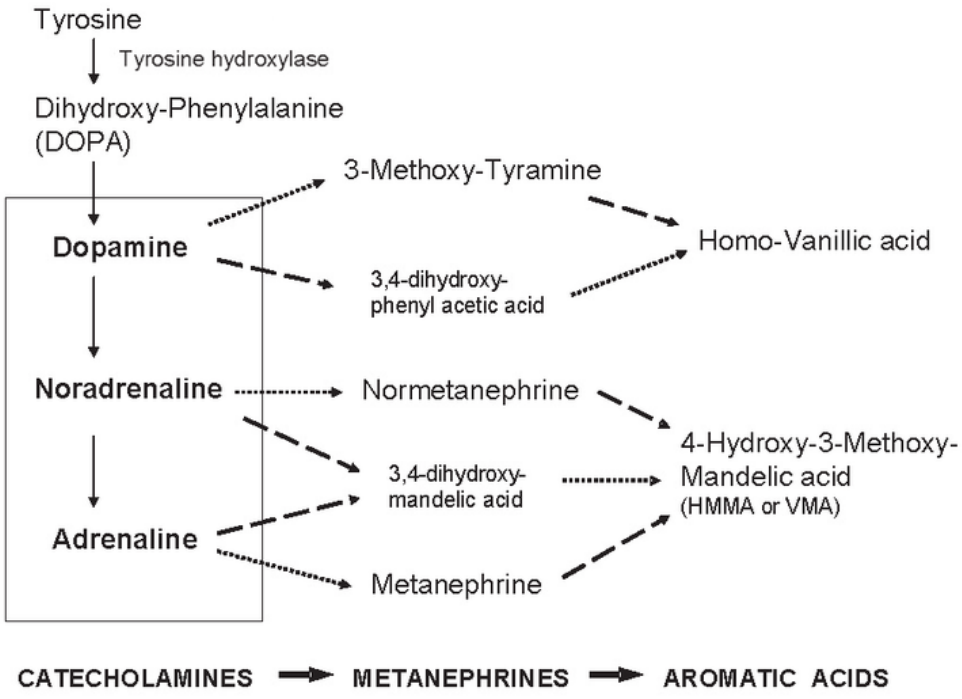

Figure 2. Degradation of monoamines. Adopted from (Whiting and Doogue 2009).

3.2 Catecholamine transport

Catecholamine transport is active and is mediated by transport proteins that belong to the solute carrier (SLC) transport family. Specifically, dopamine uptake is mediated by DAT, whereas norepinephrine transport is mediated by NET (Ganapathy, Ramamoorthy et al. 1993). These transport proteins are characterized as high-affinity and low-capacity transporters for dopamine and norepinephrine, and their function is dependent on a sodium gradient. Altered function of catecholamine transporters is associated with several neurological disorders such as depression, attention deficit hyperactivity disorder, autism or bipolar disorder. Moreover, catecholamine transporters are often a target of drugs used to treat neurological conditions, resulting in an increase in extracellular monoamines (Torres, Gainetdinov et al. 2003, De Felice 2004).

Importantly, in recent years, the high-capacity, low-affinity organic cation transporter 3 (OCT3/SLC22A3) has been suggested to mediate dopamine and norepinephrine uptake in the central nervous system (CNS) (Fraser-Spears, Krause-Heuer et al. 2019). This transporter transports catecholamines in a sodium-independent manner, however its role is not yet fully established.

3.3 Physiological functions of catecholamines

Catecholamines are important for many physiological functions in the body. There are two major pathways in the dopamine system (located in the midbrain) that are called the nigrostriatal pathway and the mesolimbic pathway. The nigrostriatal pathway is mostly connected to motor control. This pathway consists of dopamine neurons in the substantia nigra pars compacta and projects to the dorsal striatum (Liu and Kaeser 2019). The mesolimbic pathway is made of dopamine neuron somata in the ventral tegmental area, it projects to the ventral striatum (which can be also called the nucleus accumbens) and it is associated with the process of reward (Berke 2018). Dopamine can be used to treat hypotension in patients with shock. Because of its affinity for dopamine receptors found in the renal arteries, it dilates them (Paravati, Rosani et al. 2022).

One of the most important functions on norepinephrine and epinephrine is stress reaction called ”fight or flight”. They have influence on blood pressure by causing vasoconstriction in blood vessels by interacting with α_1-receptors (Akinaga, García-Sáinz et al. 2019). Enhanced cardiac muscle contractility is a result of their reaction with β_1-receptors. Norepinephrine and epinephrine also contract the pupillary dilator (α_1-receptors), cause piloerection (α_1-receptors) or relax smooth muscle of bronchioles and gastrointestinal and urinary tract (β_2-receptors). Another important function is their effect on metabolism. They increase levels of blood sugar,

which is a result of stimulation of glycogenolysis in the liver and increasing of secretion of glucagon (β_2-receptors). Another effect on metabolism is lowering of insulin secretion (α_2-receptors) and enhanced lipolysis in adipose tissue (β_3-receptors). Lastly, epinephrine is also used during anaphylactic shocks (Boden and Wesley Burks 2011).

3.4 Catecholamine importance in the prenatal period

During the prenatal period, they are among the first neurochemicals to develop in the embryo and have been shown to be essential for fetal development (Thomas, Matsumoto et al. 1995). Circulating levels of catecholamines are low during the fetal and neonatal life, increasing substantially at birth, vital for cardiovascular, pulmonary, metabolic, and endocrine adaptations during the postnatal life (Nguyen, Tseng et al. 1999). Catecholamines during the prenatal period exert multifaceted roles both in the developing fetus and in the placenta. Catecholamines are crucial for regulation of morphogenesis through cell differentiation and migration during fetal development (Hansson, Bottalico et al. 2009). Dopamine is essential for motor and cognitive functions. Dopamine and its receptors appear in the embryonic period before synaptogenesis, and data indicates the role of dopaminergic signaling for brain development (Ohtani, Goto et al. 2003). Dopaminergic neurones are formed during early fetal development, specifically during first 6-8 weeks in human (Sundström, Kölare et al. 1993). By 4 weeks of gestation, TH is detected while norepinephrine can be detected approximately by 5-6 weeks of gestation. Thanks to the norepinephrine, the Cajal-Retzius cells are the first neurones made in the cortex and are essential for laminar formation and the migration of neurons (Murrin, Sanders et al. 2007). Out of the brain, norepinephrine is also essential for fetal cardiovascular and respiratory system as well for mobilisation of glucose (Padbury 1989). Epinephrine additionally inhibits lung liquid secretion rate or causes absorption of lung liquid; lung liquid contributes to the production of amniotic fluid during pregnancy (Walters and Olver 1978).

Studies have shown dopamine and norepinephrine localization in the placenta (Zhu, Zhang et al. 2002). One of the main roles of dopamine in the prenatal period is also regulation of the production of human chorionic gonadotropin (hCG) and human placental lactogen (Belisle, Petit et al. 1992). Similarly, norepinephrine also causes increase in hCG and progesterone production that are made by the first trimester tissue (Rosenfeld 2021). Moreover, catecholamines are important during the peri-implantation period by synthesizing polyamines, interferon-τ and expression of apoptotic genes (Elmetwally, Lenis et al. 2018). Lastly, localization of D_1, D_2, and β-adrenergic receptors in the placenta suggest these may constitute

signalling pathways through which catecholamines affect placental functions (Moore and Whitsett 1982, Kim, Kim et al. 1997, Kim, Koh et al. 2001).

3.5 Placenta

The placenta is an important temporary mammalian organ during pregnancy providing the connection between mother and the fetus (Figure 3). During these nine months, the placenta plays various functions including nutrient delivery to the fetus, fetal waste excretion, and immunological protection against allosteric rejection (Staud and Karahoda 2018). The placenta also plays a big role in the fetal programming of chronic diseases (Sferruzzi-Perri and Camm 2016), which will be described later (see section 3.6).

Placental formation starts right after implantation, whereby cells undergo many division steps until the formation of the blastocyst. After several developmental events, the blastocyst differentiates into the undifferentiated type of trophoblast cells called cytotrophoblasts (CTB). CTBs are highly proliferative, and they further differentiate into i) extravillous trophoblast cells, and ii) villous multinuclear syncytiotrophoblast (STB) (Knofler, Haider et al. 2019). Structurally, the human placenta is characterized as hemochorial, with direct contact with the fetus and the umbilical cord. Maternal and fetal blood circulations are segregated by the fetal capillary endothelium and placental trophoblast (Knofler, Haider et al. 2019). Placenta consists of two major segments – maternal decidua basalis and chorionic villi. Decidua basalis is divided by decidual septa into compartments called cotyledons which serve as vascular units of the placenta. Every cotyledon is made of villous tress, covered with the multinucleated layer of STB. The STB is further polarized into two diverse membranes, specifically the microvillous membrane (facing the mother) and the basal membrane (facing the fetus). Fetal blood circulates within the chorionic villi, which is in direct contact with maternal blood. As such, the STB allows the transport of nutrients and hormones to the fetus and the excretion of waste from the fetus (Knofler, Haider et al. 2019). Additionally, STB membrane serves as a barrier for transport of exo- and endogenous substances. The surface area of the apical membrane is bigger than the area of basal membrane and also, they both consist of different receptors, transporters and enzymes (Staud and Karahoda 2018).

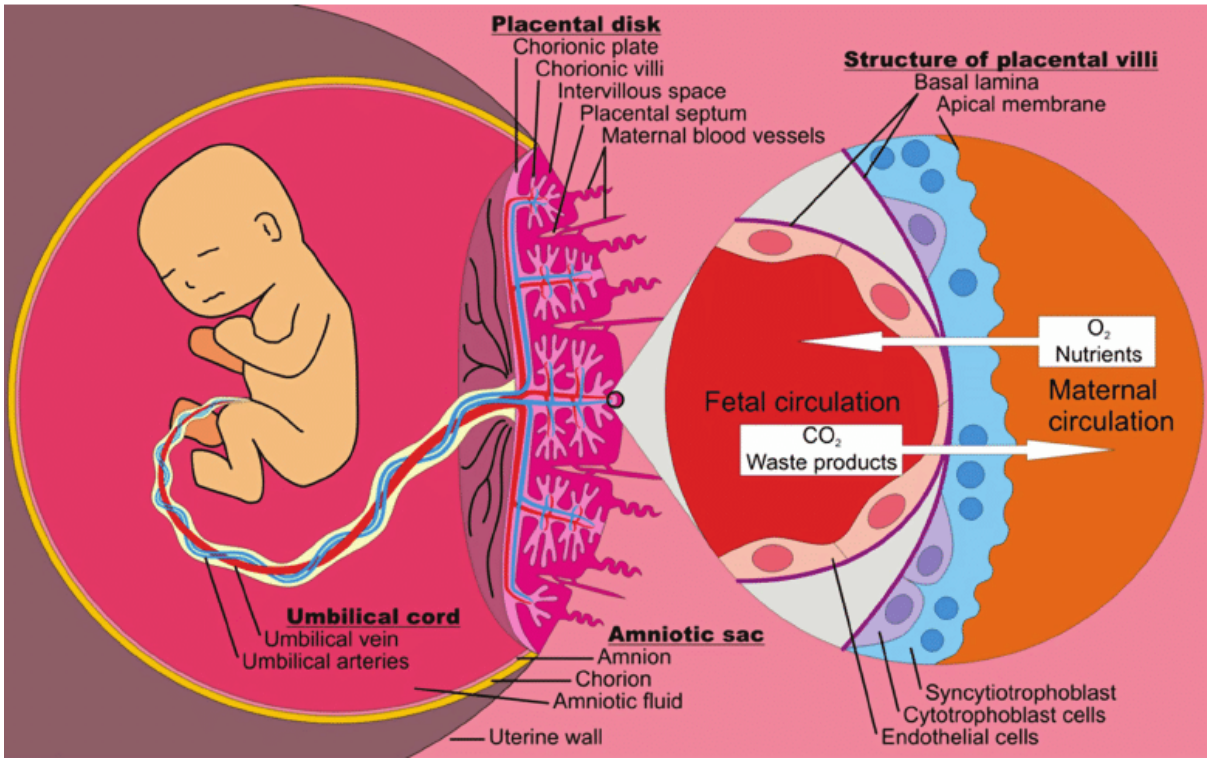

Figure 3. Structure of the placenta. Adopted from (Elad, Levkovitz et al. 2014).

3.5.1 Endocrine function

As previously described, one of the main functions of placenta is endocrine function (Napso, Yong et al. 2018). Many endogenous compounds are secreted by the placenta and are essential for the proper fetal development and programming (Staud and Karahoda 2018). Protein hormones, such as hCG or growth hormone, and steroid hormones, e.g. progesterone, are produced by STB (Chatuphonprasert, Jarukamjorn et al. 2018). hCG is one of the essential hormones which is involved in several key events during pregnancy, specifically termination of the menstrual cycle, promotion of trophoblast differentiation, and stimulation of progesterone production. Progesterone in turn promotes embryo implantation. Another class of steroid hormones produced by the placenta are the estrogens, which are crucial for the growth of fetus and milk production (Costa 2016).

Recently the placenta has been recognized also as an organ synthesizing neuroactive hormones. In 2011, Bonnin et al. described for the first time placental production of serotonin from maternally-derived tryptophan (Bonnin, Goeden et al. 2011). This was later confirmed also in primary trophoblast cells isolated from human term placenta (Laurent, Deroy et al. 2017). Such placental production of serotonin is regarded as critical early in pregnancy when the fetus is not capable of own serotonin synthesis (Bonnin and Levitt 2011). In addition, Lanoix et al. have

shown that the placenta can also synthesize melatonin (Lanoix, Beghdadi et al. 2008). Both these neuroactive hormones have been shown to regulate steroid synthesis and lactation, control maternal glucose homeostasis and importantly contribute to the development of fetal organs and programming (Napso, Yong et al. 2018, Staud and Karahoda 2018).

Importantly, in the last three years, our team has extensively investigated serotonin handling by the placenta. The study by Karahoda et al. has shown that in contrast to early pregnancy, term placenta no longer synthesizes serotonin for the fetus. Instead, term placenta is mainly responsible for the extraction of serotonin from the fetal circulation via OCT3 (Karahoda, Horackova et al. 2020). Subsequently, within the trophoblast, serotonin is metabolized by MAO-A and its metabolite 5-hydroxyindoleacetic acid is excreted to the maternal circulation via multidrug resistance-associated protein 2 (MRP2) (Staud et al., Submitted). These are very important findings suggesting that the placenta plays a role in monoamine clearance during gestation. Nonetheless, less is known about catecholamines dopamine and norepinephrine. Only one study from 1984 using human placenta perfusion showed a non-specific extraction of norepinephrine from the fetal circulation, and subsequent metabolism via MAO-A and COMT (Sodha, Proegler et al. 1984). Thus, the placenta is highly likely to be involved also in catecholamine clearance during the prenatal period.

3.5.2 Transport function

Another important function of the placenta is to bring nutrients and immunological factors to the fetus from maternal blood circulation. Transport is provided by transporters located on apical (facing the mother) and basal (facing the fetus) side of the STB (Staud and Ceckova 2015). These transporters are responsible for supplying glucose (source of energy) (Illsley 2000), amino acids (protein synthesis, source of energy) (Jansson 2001), nucleosides (Jiraskova, Cerveny et al. 2018), and fatty acids (fetal growth, brain development, fat deposition) (Lager and Powell 2012). Two main superfamilies of transporters are the solute carrier (SLC) and the ATP binding cassette (ABC) (Staud and Ceckova 2015). ABC transporters mostly carry cholesterol and they also act as efflux pumps to protect the fetus from harmful substances from the maternal blood (Vähäkangas and Myllynen 2009). SLC type of transporters provide transport of hydrophilic or charged molecules, including glucose, amino acids, monoamines etc. (Lager and Powell 2012). On the other hand, the fetus gets oxygen by passive diffusion.

When it comes to monoamine transporters in the placenta, current evidence has shown the functional expression of SERT/SLC6A4 and NET, however, DAT is reportedly not expressed (Ganapathy, Ramamoorthy et al. 1993). NET is responsible for uptake of dopamine and norepinephrine, while SERT contributes to uptake of serotonin (Balkovetz, Tiruppathi et al. 1989, Ramamoorthy, Leibach et al. 1992, Ramamoorthy, Prasad et al. 1993). OCT3 transporter, which has low affinity and high capacity to non-selective transport of monoamines, has been reported by our team to be expressed in basal membrane and to carry out the transport and uptake of serotonin (Karahoda, Horackova et al. 2020). The importance of this transport in placental transfer of dopamine and norepinephrine is yet to be determined.

3.6 Catecholamines as intermediates in fetal programming of adulthood disease

If any of the placental functions are impaired during pregnancy, it can result in altered fetal development and programming (Figure 4). The fetus can adapt to suboptimal conditions in order to survive, however this permanently changes its metabolism and physiology, leading to long-term structural and functional changes within the fetus (Sferruzzi-Perri and Camm 2016, Staud and Karahoda 2018). The concept of fetal programming was first mentioned by D.J. Barker, who reported connections between the size of placenta and the fetus and the likelihood of developing cardiovascular, CNS and metabolic disorders in adulthood (Barker, Bull et al. 1990, Barker, Godfrey et al. 1993). With time, several studies have shown that insults during pregnancy affect placental function and in turn fetal programming.

In particular, prenatal monoamine neurotransmitter handing has been implicated with several neurodevelopmental and neurobehavioral conditions (Goeden, Velasquez et al. 2013, Rosenfeld 2021). Current research focus is aimed at understanding mechanistic links between monoamines and fetal programming of mental health. Several lines of research indicate that altered monoamine homeostasis is linked to an increased risk of neurological disorders, such as schizophrenia, depression, autism, bipolar affective, or attention deficit hyperactivity disorder (Robinson, Schutz et al. 2001, Previc 2007, Saboory, Ghasemi et al. 2020). Stress, through its effects on glucocorticoid system, has been shown to in turn cause changes in the content of neurotransmitters in the fetal brain (Kurosawa, Kageyama et al. 1980). Prenatal stress is also linked to higher risk of development of neurological diseases such as schizophrenia (Herlenius and Lagercrantz 2001). Depression is also thought to generate as consequence of fetal programming in connection to changes in prenatal serotonin levels (O'Donnell and Meaney 2016). Melatonin also plays its role in the programming to preserve the fetus from hypoxia (Sagrillo-Fagundes, Assunção Salustiano et al. 2018). One of the most common hypotheses of

developing schizophrenia is imbalance of dopamine levels. Several animal studies found out that prenatal inflammation or infection leads to impairment of dopaminergic structures (Aguilar-Valles, Flores et al. 2010, Eyles, Feldon et al. 2012). Disbalances in norepinephrine levels can lead to developing of hypertension (Vieira-Rocha, Rodríguez-Rodríguez et al. 2019)

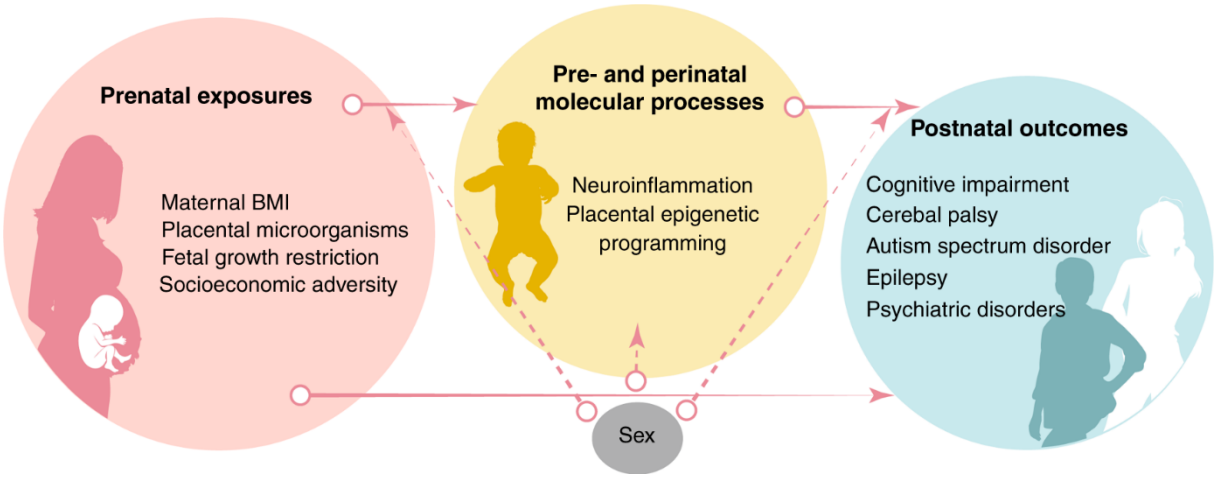

Figure 4. The link between prenatal homeostasis and fetal programming of adult diseases. Adopted from (Bangma, Hartwell et al. 2021).

4 AIM OF STUDY

Several lines of evidence support the notion that the placenta represents a source and regulator of catecholamines levels in the fetoplacental unit (Rosenfeld 2021). However, controversies remain on the placental expression of proteins involved in catecholamine handling.

Thus, the aim of this thesis was to:

- Analyze the gene expression of catecholamine system in human placental cell-based models *in vitro* (BeWo, JEG-3, primary human trophoblast (PHT) cells)
- Investigate the effects of trophoblast differentiation on pathway expression in BeWo and PHT cells
- Evaluate the expression of enzymes and transporters involved in catecholamine homeostasis in the human and rat placenta during gestation
- Assess the expression of enzymes and transporters involved in catecholamine homeostasis in rat fetal organs (brain, intestine, liver, kidneys, lungs, and heart)

5 METHODOLOGY

5.1 Sample cohort

Human placenta samples belong to a tissue cohort collected at the University Hospital in Hradec Králové, Czech Republic and used in our team‘s previous study (Karahoda, Abad et al. 2020). Samples of first-trimester placenta were obtained from elective interruptions of healthy pregnancies. On the other hand, term placentas were obtained from uncomplicated pregnancies after delivery. Patient characteristics for the samples can be found in the original publication (Karahoda, Abad et al. 2020). All the samples were obtained after the women signed the informed consent with the approval of the University Hospital Research Ethics Committee (201006 S15P).

Similarly, rat placental tissues and fetal organs were obtained from Wistar rats, a cohort previously used by Abad et al. (Abad, Karahoda et al. 2020). Samples consisted of different gestational ages, specifically gestation day (GD) 15, 18 and 21. Fetal organs comprised the brain, intestine, liver, heart, kidneys, and lung. These experiments were approved by the Ethical Committee of the Faculty of Pharmacy in Hradec Králové (MSMT-4312/2015-8).

5.2 Cells

The BeWo and JEG-3 choriocarcinoma cells were received from the European Cell Culture Collection (United Kingdom), while the BeWo b30 cell line was obtained from Dr. Christiane Albrecht, University of Bern, Switzerland (with permission from Dr. Alan Schwartz, Washington University, USA). BeWo cells were cultured in Ham F-12 medium supplemented with 10% FBS, JEG-3 cells were cultured in MEM supplemented with 10% FBS, and BeWo b30 cells were cultured in DMEM (high glucose) supplemented with 10% FBS. The medium was changed every day and the cells were cultured at 37°C, 5% CO2, 95% N2.

On the other hand, samples (RNA) from primary trophoblast cells isolated from human term placenta were obtained from our team’s collection and were directly used for further analysis.

5.3 RNA isolation

To isolate ribonucleic acid (RNA) from cells, the culture medium was aspirated and 1 ml TRI Reagent (Molecular Research Center, USA) per 10 cm^2 of culture dish was added. This mixture was allowed to stand in incubator for 5 minutes and then collected for RNA isolation. On the other hand, the tissues were cut into 100 mg fragments, washed in physiological solution (PBS)

and then dried by using a gauze. After that, 1 ml of TRI Reagent per 100 mg tissue was added and the tissues were homogenized twice for 20 seconds.

In the next step, chloroform was added to samples and the tubes were shaken up and down for at least 15 seconds. The samples were then centrifuged at 12,000 g for 15 minutes at 4°C to separate the mixture into three phases (Figure 5). The aqueous phase (upper phase) was put into new Eppendorf tube without disturbing the other phases. To precipitate the RNA, 2-propanol was added to samples, the tubes were shaken and let to stand for 10 minutes in room temperature. The samples were then mixed and centrifuged at 12,000 g for 10 minutes at 4°C. The resulting RNA precipitate appeared as white pellet on the wall of the tube (Figure 5).

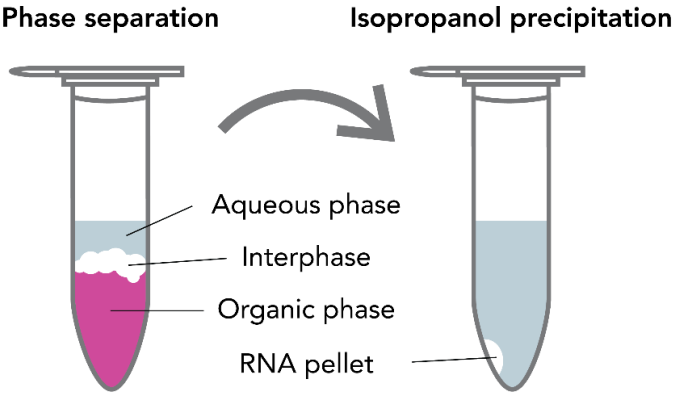

Figure 5. Phase separation and RNA precipitation

The supernatant was removed, and the RNA was washed twice by using 75% ethanol and centrifugation at 12,000 g for 5 minutes at 4°C. Finally, the ethanol was aspirated and allowed to evaporate. The pellet was dissolved in aqua pro injection (API).

The spectophotometer NanoDrop™ 1000 (Thermo Fisher Scientific, USA) was used to measure the concentration and purity of RNA. 1 µl of RNA was added to Nanodrop and the absorbance was measured at 230, 260 and 280 nm. The concentration of RNA was calculated based on the absorbance value at 260 nm and was reported in ng/µl. On the other hand, absorbance ratios reflected RNA purity. Absorbance ratios 260/280 (optimal between 1.8 and 2.2) were used to exclude contamination by protein and DNA, whereas the 260/230 ratio (optimal greater than 1.7) contamination by organic solvents.

5.4 Reverse transcription

iScript Advanced cDNA Synthesis Kit (Bio-Rad, United States) was used to perform reverse transcription of isolated RNA. 5,000 ng RNA were transcribed in a 20 µl reaction on a Bio-Rad

T100^{TM} thermal cycler. Reverse transcription was conducted for 20 minutes at 46°C, followed by a transcriptase inactivation step of 1 minute at 95°C. All these steps were performed according to the manufacturer's instructions. The obtained cDNA (250 ng/µl) was diluted to 12.5 ng/µl prior to use in PCR analysis.

5.5 Quantitative PCR analysis

Quantitative polymerase chain reaction (qPCR) is an efficient method used to investigate gene expression in a specific tissue/cell type. 12.5 ng/µl were amplified using the TaqMan Universal Master Mix II, no UNG (Thermo Fisher Scientific, USA) and predesigned TaqMan Assays (Thermo Fisher Scientific, USA) in total 5 µl reactions. Table 1 lists all the primers used in the thesis. Thermal conditions included a polymerase activation step at 95°C for 10 minutes. Subsequently, 40 steps of denaturation and annealing were carried out at 95°C (15 seconds) and 60°C (1 minute), respectively. Amplification was performed in QuantStudio 6 (Thermo Fisher Scientific, USA). The obtained Ct values were used to calculate the gene expression. Two reference genes were used for normalizations (in humans - *GAPDH* and *B2M*; in rats - *Gapdh* and *Ywhaz*; see table 1 for assay ID).

Table 1. List of TaqMan® gene assays used in the thesis. All assays were obtained from Thermo Fisher Scientific (USA).

Gene name	**Gene symbol**	**Assay ID**
Human tissues/cells		
tyrosine hydroxylase	*TH*	Hs00165941_m1
DOPA decarboxylase	*DDC*	Hs01105048_m1
dopamine β-hydroxylase	*DBH*	Hs01089840_m1
phenylethanolamine-N-methyltransferase	*PNMT*	Hs01557113_g1
catechol-O-methyl transferase	*COMT*	Hs00241349_m1
norepinephrine transporter	*NET/SLC6A2*	Hs00426573_m1
dopamine transporter	*DAT/SLC6A3*	Hs00997374_m1
glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	Hs02786624_g1
β-2-microglobulin	*B2M*	Hs00187842_m1
Rat tissues		
tyrosine hydroxylase	*Th*	Rn00562500_m1
DOPA decarboxylase	*Ddc*	Rn01401189_m1
dopamine β-hydroxylase	*Dbh*	Rn00565819_m1
phenylethanolamine-N-methyltransferase	*Pnmt*	Rn01495589_g1
catechol-O-methyl transferase	*Comt*	Rn01404927_g1
norepinephrine transporter	*Net/Slc6a2*	Rn00580207_m1
dopamine transporter	*Dat/Slc6a3*	Rn00562224_m1
glyceraldehyde 3-phosphate dehydrogenase	*Gapdh*	Rn01775763_g1
tyrosine 3-Monooxygenase/tryptophan 5-monooxygenase activation protein zeta	*Ywhaz*	Rn00755072_m1

5.5.1 Calculation of gene expression

The $2^{\Delta\Delta Ct}$ method was used to calculate the relative normalized expression of genes, using the two formulas below, obtained from CFX Maestro Software User Guide (Bio-Rad, USA):

$$Relative\ Quantity_{sample} = 2^{(Ct\ \text{average} - Ct\ sample)}$$

$$Normalized\ Expression = \frac{Relative\ Quantity_{target\ gene}}{geometric\ mean\ of\ Relative\ Quantity_{reference\ gene}}$$

Since we were working with a cohort of samples where there was no 'control' sample, relative quantification was performed against the average Ct value of all samples for a given target gene.

5.6 Western blotting

Aliquots of tissue protein used in this thesis were obtained from isolated protein homogenates used in previous publications (Abad, Karahoda et al. 2020, Karahoda, Abad et al. 2020). To 30-70 µg of protein homogenates 4x Laemmli Sample Buffer was added (BioRad, USA; 1x final concentration supplemented with 10% β-mercaptoethanol) and the samples were heated for 5 minutes at 96°C. Proteins were separated by SDS-PAGE on polyacrylamide gels, then the electrophoresis was conducted at 120 V. In the next step, proteins were transferred to polyvinylidene fluoride (PVDF) membranes (BioRad, USA), and afterwards blocked for 1 hour at room temperature in 5% BSA in TBS-T (20 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween 20). The membranes underwent three rounds of washing and consequently incubated with secondary antibodies at 4°C overnight. The used antibodies are listed in Table 2. The following day, the membranes were incubated with anti-rabbit secondary antibody (P0217, 1:20000; Dako, USA) for 1 hour and room temperature after washing with TBS-T buffer and then the washing was performed again. With the use of using Amersham™ ECLTM Prime (GE Healthcare Life Science, USA) the membranes were developed. To quantify the bands the densitometric analysis was carried out using ChemiDoc™ MP, Imaging system (Bio-Rad, USA). Membranes were examined for β-actin (see Table 2 for details) and specific secondary antibody (P0260, 1:20000; Dako, USA) to ensure the corresponding loading of proteins.

Table 2. List of antibodies used in the thesis.

Protein name	**Protein symbol**	**Catalogue number**	**Dilution**	**Gel**	**Supplier**
tyrosine hydroxylase	TH	AB152	1:1000	10%	Merck, USA
DOPA decarboxylase	DDC	AB1569	1:500	12.5%	Merck, USA
dopamine β-hydroxylase	DBH	PA5-34664	1:1000	10%	Invitrogen, USA
norepinephrine transporter	NET/ SLC6A2	Ab41559	1:500	10%	Abcam, UK
dopamine transporter	DAT/ SLC6A3	D6944	1:1000	10%	Sigma Aldrich, USA
β-actin	β-actin	Ab8226	1:10000	-	Abcam, UK

5.7 Statistical analysis

Statistical analyses were implemented in GraphPad Prism 9.3.1 software (GraphPad Software, Inc.). Statistical analysis between CTB and STB was performed using unpaired t-test. Human placental tissue was evaluated using non-parametric Mann-Whitney test. Rat placenta experiments were analysed using parametric One-Way ANOVA, whereas fetal organs by parametric Student's t-test. Significance levels were calculated: *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$.

6 RESULTS

6.1 Gene expression of catecholamine pathway in human placental *in vitro* models

We evaluated the gene expression of *TH, DDC, DBH, PNMT, COMT, NET*, and *DAT* in three choriocarcinoma cell lines: BeWo b30 clone, BeWo, and JEG-3. Additionally, we analysed the spectrum of enzymes and transporters expressed in PHT cells. We observed that while BeWo b30, BeWo, and JEG-3 cells express *TH* (Figure 6A) and *DAT* (Figure 6F), PHT cells lack expression at mRNA level. On the other hand, at the basal level, JEG-3 cells did not express *PNMT* (Figure 6C). *DDC*, *COMT*, and *NET* was expressed in all in vitro models tested (Figure 6B, 6D, 6E). Importantly, none of the cells expressed *DBH*.

In addition, we observed certain alterations in the gene expression profile associated with trophoblast differentiation. Specifically, trophoblast differentiation stimulated by forskolin treatment in BeWo cells significantly upregulated the expression of *DDC*, *COMT*, *NET*, and *DAT* (Figure 6B, 6D, 6E, 6F). On the other hand, *PNMT* expression in BeWo b30 was undetectable upon syncytialization (Figure 6C). In contrary, spontaneous trophoblast differentiation in PHT cells did not induce transcriptional changes in any of the enzymes/transporters tested.

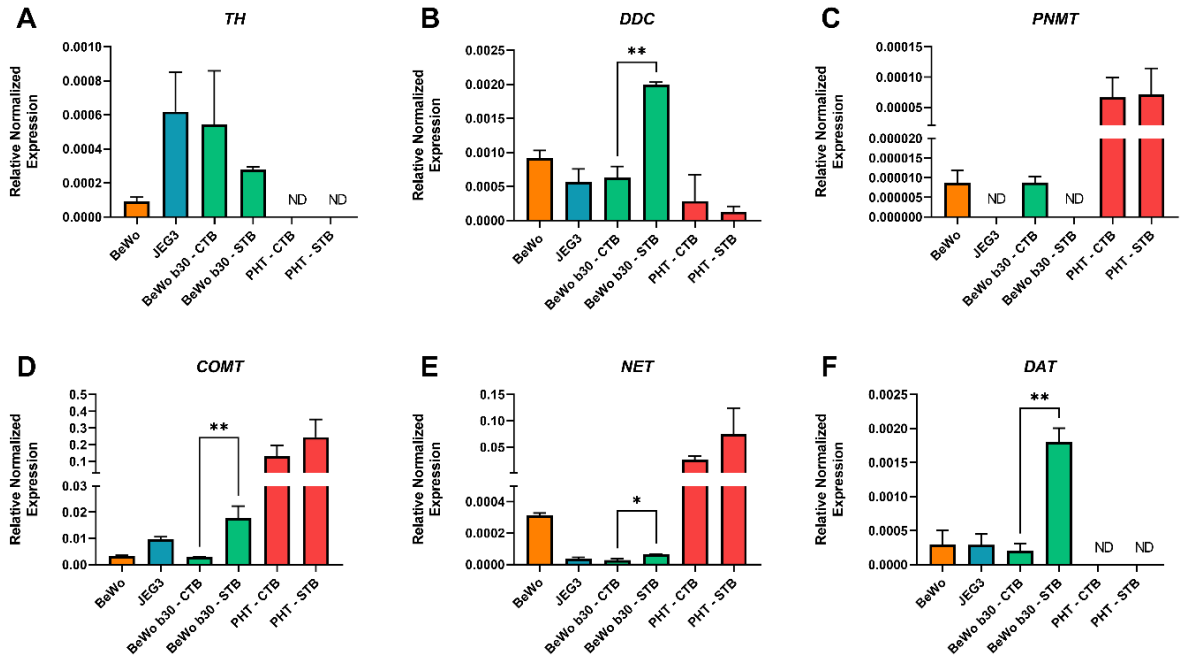

Figure 6. Relative normalized expression of catecholamine genes in placental cells. The expression of catecholamine synthesizing (A, B, C) and degrading (D) enzymes, and transporters (E, F) was investigated in BeWo b30, BeWo, JEG-3 and PHT cells. Additionally, the effect of trophoblast differentiation on mRNA expression was evaluated in BeWo b30 and

PHT cells. Data are shown as mean + SD. Statistical analysis between CTB and STB was performed using unpaired t-test. N = 3. *p ≤ 0.05, **p ≤ 0.01. ND - not determined.

6.2 Gestational-age dependent changes in the gene expression of catecholamine pathway in the human placenta

Gene expression of *TH, DDC, DBH, PNMT, COMT, NET*, and *DAT* was evaluated in first trimester (gestational age 8-11 weeks) and term (gestational age 38-40 weeks) human placenta. Of all genes tested, only *PNMT*, *COMT* and *NET* were expressed in the human placenta tissue (Figure 7). Importantly, the expression of all these genes significantly increased during gestation, with the most significant upregulation observed for *PNMT* and *NET* (Figure 7A, 7C).

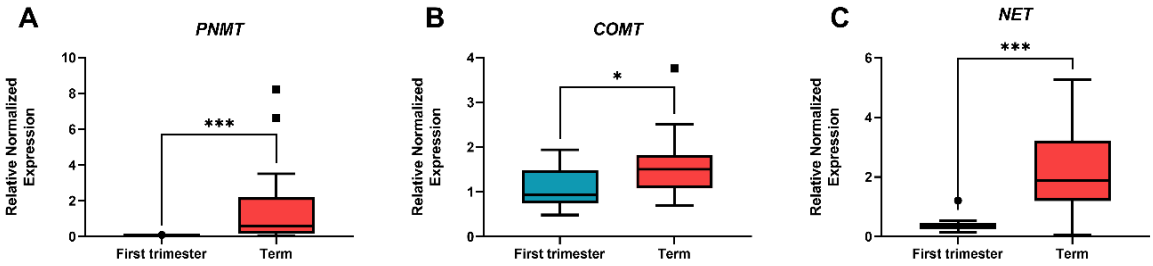

Figure 7. Gestational-age dependent gene expression of catecholamine enzymes and transporters in the human placenta. The only genes expressed in the human placental tissue include *PNMT* (A), *COMT* (B), and *NET* (C). *TH, DDC, DBH*, and *DAT* expression was not detected. Data are shown as Tukey plots. Statistical analysis was performed using Mann-Whitney test. N ≥ 13. *p ≤ 0.05, ***p ≤ 0.001.

6.3 Gene and protein expression of catecholamine enzymes and transporters in rat placenta during mid-to-late gestation

Gene expression of *Th, Ddc, Dbh, Pnmt, Comt, Net*, and *Dat* was evaluated in the rat placenta at GD 15, 18 and 21. Moreover, we also examined the expression levels at GD 12, however, at this stage the placenta cannot be fully separated from the fetus. Therefore, as it includes a mixture of placental and fetal tissue, these results are treated separately and are not included in the statistical analysis against other gestational periods. Interestingly, compared to the human placenta, in rats we observed mRNA expression of all the genes involved in catecholamine metabolic pathway (Figure 8A-G). Additionally, we observed significant gestational-age

dependent changes as the pregnancy proceeded from mid-to-late gestation. Specifically, the expression of *Th* was highest at GD 15, decreasing significantly towards term (Figure 8A). Similarly, *Dat* expression was downregulated at GD 18 and 21 (Figure 8G). In contrary, the expression of catecholamine metabolizing enzyme *Comt*, was significantly upregulated at GD 18 and 21 (Figure 8E).

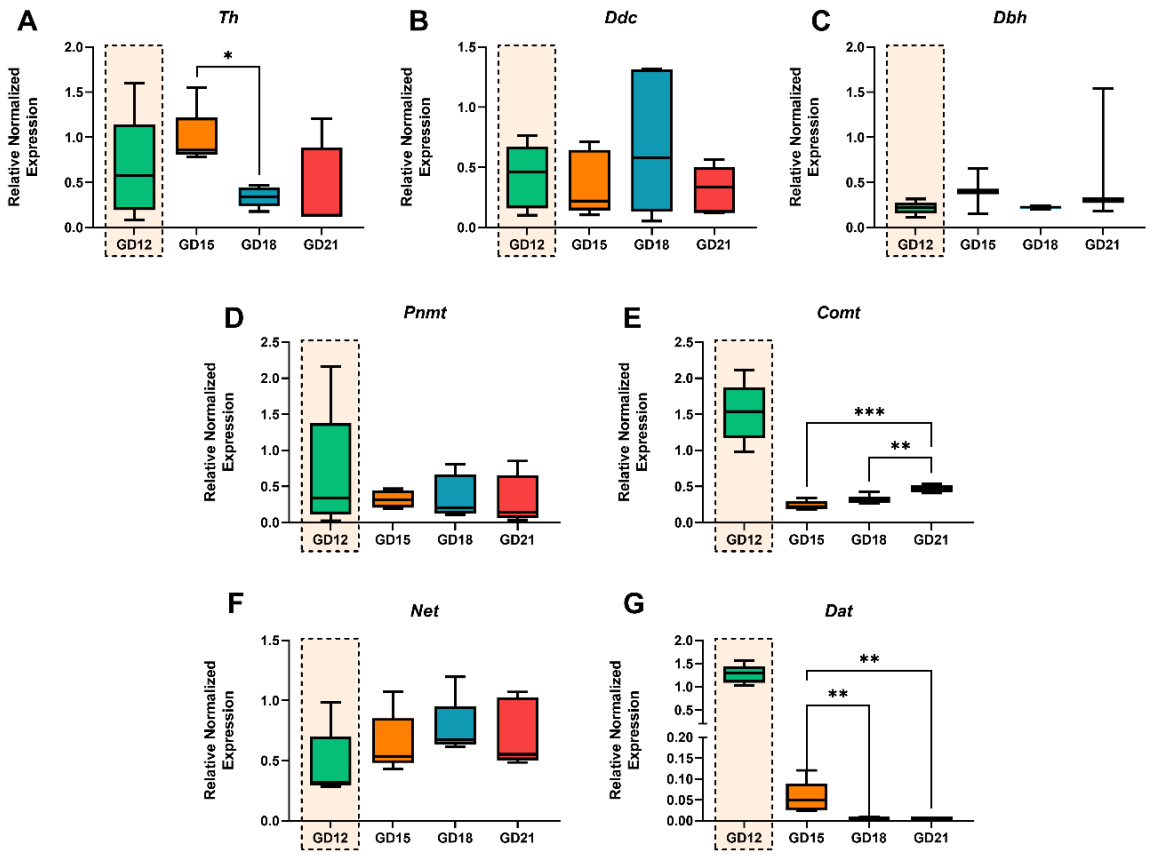

Figure 8. Gestational-age dependent gene expression of catecholamine enzymes and transporters in the rat placenta. Rat placenta expresses all the genes involved in catecholamine synthesis, metabolism and transport. While the expression of *Ddc* (B), *Dbh* (C), *Pnmt* (D), and *Net* (F) did not change during gestation, *Th* (A), *Comt* (E), and *Dat* (G) expression was significantly regulated during the progression of pregnancy. Data are shown as mean + SD. Statistical analysis was performed using One-Way ANOVA. N = 5. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. GD 12 was excluded from statistical comparison.

Next, we aimed to evaluate the protein expression of selected catecholamine enzymes and transporters. In this thesis, we evaluated the expression of TH, DDC, DBH, NET and DAT at

GD 15, 18 and 21. Consistent with the mRNA results (Figure 8), we observed expression of all proteins tested (Figure 9A-E). Moreover, we found that TH expression (Figure 9A) shows a tendency towards downregulation from GD 15 to 21 (p value = 0.0571). On the other hand, DDC expression (Figure 9B) was more expressed at term (p value = 0.0933). Significantly affected proteins included DBH (Figure 9C) and NET (Figure 9D), whose protein was expressed in higher amounts at GD 15. DAT expression showed no alterations during gestation (Figure 9E). Representative blots of target genes and beta-actin as the normalizing protein are shown in Figure 9F.

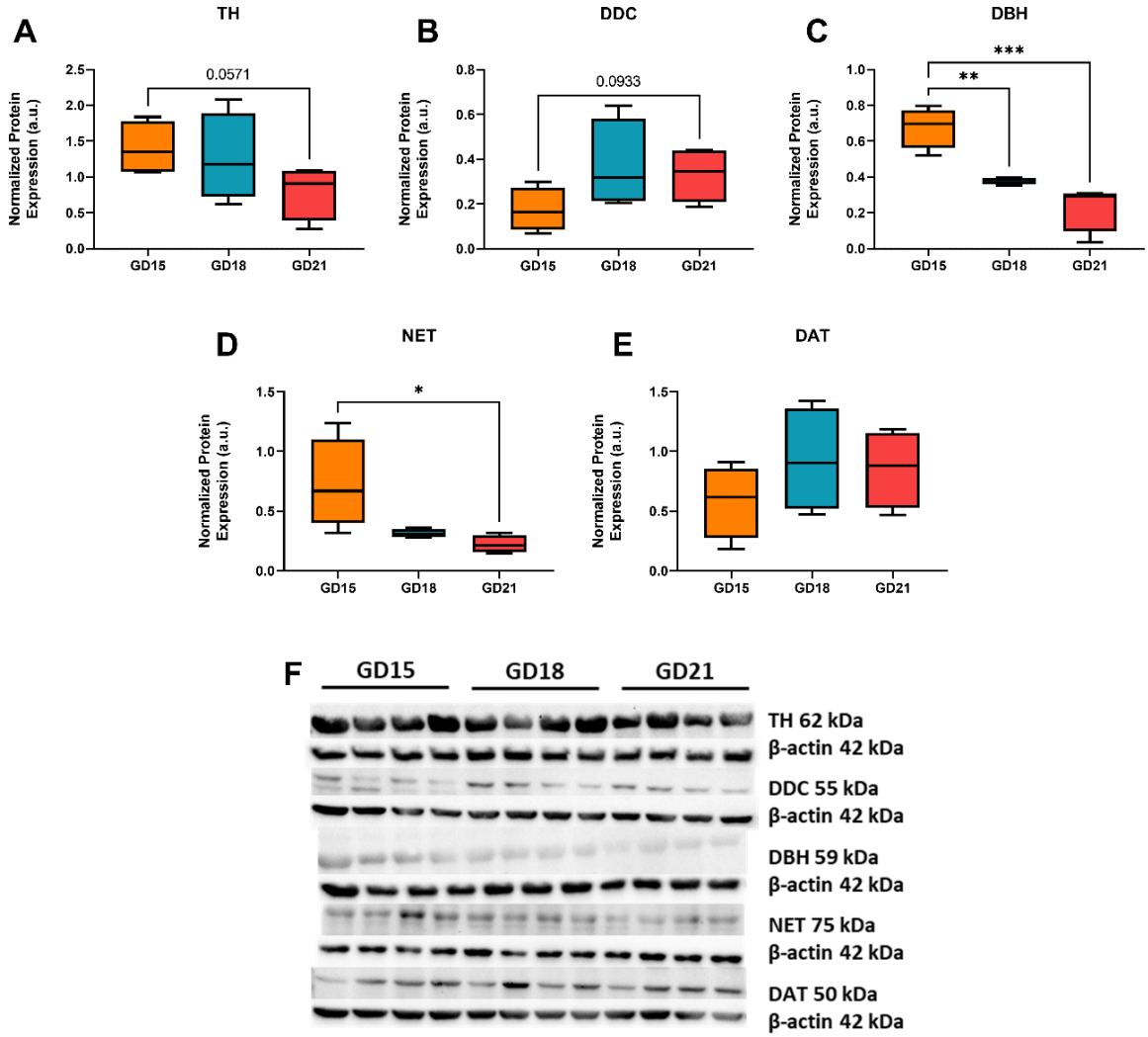

Figure 9. Protein expression of catecholamine enzymes and transporters in the rat placenta at GD 15, 18, and 21. Rat placenta expresses all the genes involved in catecholamine synthesis, metabolism and transport. TH (A), DDC (B), DBH (C), and NET (D) expression was significantly regulated during the gestation. On the other hand, DAT (E) expression was unaltered. Target protein expression was normalized against beta-actin, as loading control.

Representative blots are further shown (F). Data are shown as mean + SD. Statistical analysis was performed using One-Way ANOVA. N = 4. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

6.4 Expression of catecholamine system in rat fetal organs at late gestation

Gene expression of *Th, Ddc, Dbh, Pnmt, Comt, Net*, and *Dat* was evaluated in the fetal brain, intestine, liver, kidneys, lungs, and heart at GD 18 and 21. We observed expression of all genes in the organs evaluated (Figure 10A-F). Several genes were regulated during the organ maturation from GD 18 to 21. Specifically, in the fetal brain, we observed downregulation of *Dbh* and *Pnmt* at term (Figure 10A). On the other hand, the fetal intestine was associated with downregulated *Th*, *Dbh*, and *Net*, and upregulated *Comt* at GD 21 (Figure 10B). In the fetal liver, *Comt* and *Net* were significantly upregulated at term (Figure 10C). *Pnmt* expression was significantly at GD 18 in the fetal kidneys (Figure 10D), whereas in the lungs it was upregulated at term (Figure 10E). In the fetal heart, we observed no regulation of genes tested (Figure 10F).

To evaluate whether the fetal organs also express these enzymes and transporters at a protein level, Western blot analysis was carried out at GD 21, using two representative samples from each organ. Figure 10F shows and confirm that all organs evaluated express DAT, DBH, DDC, NET, and TH at protein level. PNMT and COMT protein expression was not evaluated.

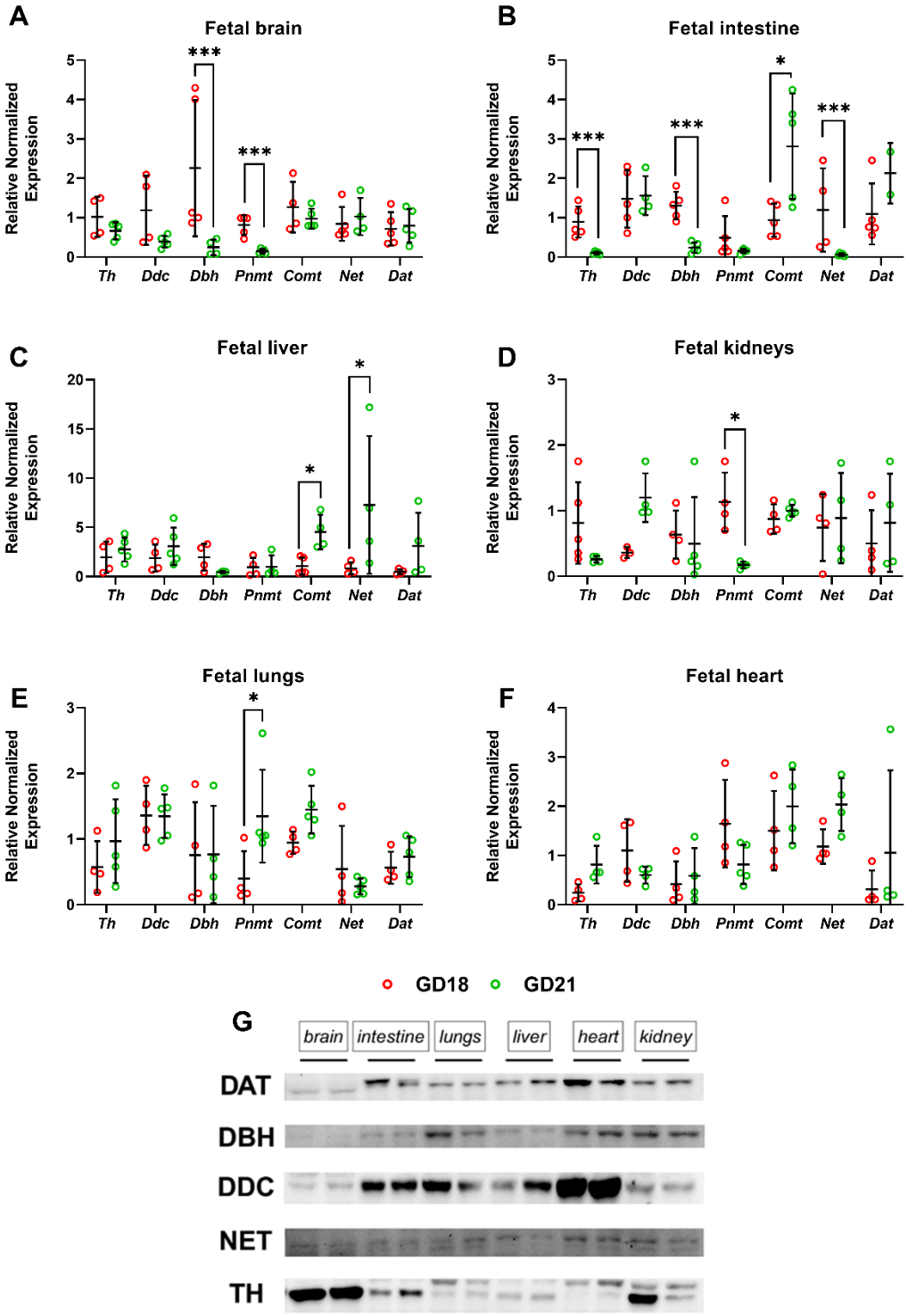

Figure 10. Protein expression of catecholamine enzymes and transporters in rat fetal organs at GD 18 and 21. Fetal brain (A), intestine (B), liver (C), kidneys (D), lungs (E), and heart (F) express all the genes involved in catecholamine synthesis, metabolism, and transport. Moreover, Western blot analysis of DAT, DBH, DDC, NET, and TH confirmed expression also at the protein level (F). Data are shown as mean + SD. Statistical analysis was performed using Student's t-test. $N \geq 4$ (except in the protein data $N = 2$). *$p \leq 0.05$, ***$p \leq 0.001$

7 DISCUSSION

In this thesis, we aimed to investigate the expression of enzymes and transporters involved in the synthesis, metabolism, and transport of dopamine and norepinephrine, two critical catecholamines for placental and fetal development and programming. The thesis was conducted in selected placental *in vitro* models, human and rat placenta during gestation, and rat fetal organs at the end of pregnancy. We identified the expression of several components of catecholamine pathway, however, there were significant differences in the spectrum of expression between different models.

When it comes to placental *in vitro* models, current knowledge is limited on the expression and functional activity of catecholamine pathways in cells. However, it is important to identify the most suitable cellular model as they are useful in the initial determination of several physiological and pathological aspects of placental functioning. The most used placental cell lines are BeWo and JEG-3 coming from first-trimester choriocarcinoma (Rothbauer, Patel et al. 2017). On the other hand, PHT cells can be isolated from human term placenta; however, their use is limited as they are nonproliferative and the isolation procedure is demanding. Here, we report significant differences in the expression of several genes involved in catecholamine synthesis and transport across healthy and choriocarcinoma cells. Importantly, the expression in PHT is highly similar to that observed in healthy human placenta. Previous studies have also shown distinct differences between choriocarcinoma and primary cells (Azar, Valentine et al. 2018, Kallol, Moser-Haessig et al. 2018, Karahoda, Abad et al. 2020). The most notable difference was observed in the case of *TH* and *DAT* expression, which were not expressed in PHT cells, while in BeWo and JEG-3 cells their expression was significant. This may represent an advantageous mechanism by choriocarcinoma cells for dopamine synthesis and transport, as dopamine is an important differentiation and proliferation agent (Popolo, McCarthy et al. 2004). Moreover, while in PHT cells trophoblast differentiation did not induce any changes in the expression of genes, in BeWo cells this effect was more profound. Thus, these results point out to be careful in selecting *in vitro* models for future studies.

In 2011, Bonnin et al. reported for the first time that the placenta contributes to serotonin levels in the mouse fetoplacental unit during pregnancy (Bonnin, Goeden et al. 2011). Subsequently, this was confirmed also in PHT cells isolated from the human term placenta (Laurent, Deroy et al. 2017). These findings raised the exciting possibility that the placenta may also influence dopamine and norepinephrine homeostasis in the prenatal stage (Rosenfeld 2021). Thus, here we aimed to screen the expression signature of catecholamine pathway in the human and rat

placenta. Our teams' previous studies on tryptophan metabolism revealed similar patters of expression in the human and rat placenta (Abad, Karahoda et al. 2020, Karahoda, Abad et al. 2020), thus making the rat model as an ideal alternative to human research for tryptophan metabolism. However, when it comes to dopamine levels in the fetoplacental unit, there are reported differences between humans and rats which should be considered. While in rats dopamine dominates in the fetal circulation in comparison to maternal circulation (Ben-Jonathan and Maxson 1978), in human the levels are similar (Peleg, Munsick et al. 1986). This may, at least partially, be explained by the differences in the expression of catecholamine enzymes and transporters expression in the human and rat placenta we observed in this thesis. The rat placenta expresses TH and DBH, both at gene and protein level, while human placenta lacks the gene expression of these enzymes essential for dopamine synthesis. However, our qPCR results from human placenta contradict two previous reports where the authors determined TH, DDC, and DBH expression in the human placenta and PHT cells (Manyonda, Slater et al. 1998, Siaterli, Vassilacopoulou et al. 2003). Therefore, more studies at protein and functional level are necessary to identify the potential of the human placenta to synthesize dopamine. Importantly, in rats we observed that decreased expression of the rate-limiting enzyme TH from GD 15 to 18. These findings could explain the decline in dopamine levels observed in rats towards delivery (Ben-Jonathan and Maxson 1978).

Previous research indicates that the placenta contributes to fetal catecholamine clearance (Bzoskie, Blount et al. 1995). Transporters as well as metabolizing enzymes (MAO-A and COMT) are responsible for this phenomenon by taking up catecholamines from both maternal and fetal circulation and by metabolizing catecholamines, respectively. In a previous study, our team observed upregulated MAO-A expression during pregnancy (Abad, Karahoda et al. 2020, Karahoda, Abad et al. 2020). Here we show that also COMT expression is highly increasing in both human and rat placenta towards term. NET expression increases towards delivery as well, which indicates an interplay in both transporters and metabolizing enzymes in mediating catecholamine clearance towards term.

Apart from the placenta, in this thesis we also evaluated the expression of catecholamine pathway in selected fetal organs. As this cannot be done in humans due to ethical limitations, we selected the Wistar rat model. We report that all examined enzymes and transporters involved in catecholamine homeostasis are expressed in rat fetal brain, intestine, liver, kidneys, heart, and lungs. Expression of enzymes involved in catecholamine synthesis in the fetal heart corresponds to previous findings of dopamine and norepinephrine synthesis upon perfusion

with tyrosine (Gennser and Von Studnitz 1975). Moreover, we observed increased fetal heart *Th* expression towards the delivery. If confirmed at the functional level, this could suggest upregulated synthesis of norepinephrine and epinephrine towards term as they are highly important in heart rate control (Ebert and Taylor 2006). On the other hand, in the fetal liver, we observed an upregulation of *Comt.* As the liver constitutes the organ with the highest COMT activity (Axelrod 1971), increased expression at term could indicate potential organ maturation and preparation for life after birth.

Expression of catecholamine pathway has several functions in essential organs such as the lungs or the brain. Dopamine is critical in the process of lung liquid reabsorption at birth (Chua and Perks 1998), thus the expression of enzymes involved in dopamine synthesis in the lungs is important during the end of pregnancy. Moreover, catecholamines are essential for brain development. Our findings of fully present enzymatic machinery in the fetal brain are in agreement with functional findings of increased dopamine and norepinephrine levels in rat fetal brain upon administration of tyrosine to pregnant rats (Arevalo, Castro et al. 1987, Garabal, Arévalo et al. 1988). Similarly, our findings in the fetal intestine are further supported by previous studies of substantial TH activity and functional dopamine synthetic pathway in the gastrointestinal tract (Eisenhofer, Aneman et al. 1997).

In summary, this thesis was designed as a hypothesis-generating study to provide an overview of catecholamine pathways in the fetoplacental unit. While this thesis was conducted on a well-characterized cohort of samples from early to late gestation, it is limited mainly at the gene and partially at protein expression level. Thus, future studies should be focused in analyzing the protein and functional relevance of these enzymes and transporters. Understanding the fetoplacental catecholamine pathways under physiological conditions is critical in identifying mechanisms involved in fetal programming. All these genes/proteins are target of various pharmacological agents including antidepressant drugs (Horackova, Karahoda et al. 2021). Moreover, catecholamines may represent important signals in various pregnancy pathologies.

8 CONCLUSIONS

In conclusion, we suggest that during pregnancy, regulatory pathways control levels of norepinephrine and dopamine in the fetoplacental unit to ensure proper embryo and fetal development throughout gestation. Our data also indicate significant differences between *in vitro* placental models, which should be taken into consideration when performing research on catecholamine pathways. Moreover, we provide important aspects on interspecies differences between humans and rats, which question the suitability of the rat model as an alternative for studies on catecholamine synthesis.

9 REFERENCES

Abad, C., R. Karahoda, P. Kastner, R. Portillo, H. Horackova, R. Kucera, P. Nachtigal and F. Staud (2020). "Profiling of Tryptophan Metabolic Pathways in the Rat Fetoplacental Unit During Gestation." Int J Mol Sci **21**(20).

Aguilar-Valles, A., C. Flores and G. N. Luheshi (2010). "Prenatal inflammation-induced hypoferremia alters dopamine function in the adult offspring in rat: relevance for schizophrenia." PLoS One **5**(6): e10967.

Akinaga, J., J. A. García-Sáinz and A. S Pupo (2019). "Updates in the function and regulation of α(1) -adrenoceptors." British journal of pharmacology **176**(14): 2343-2357.

Arevalo, R., R. Castro, M. D. Palarea and M. Rodriguez (1987). "Tyrosine administration to pregnant rats induces persistent behavioral modifications in the male offspring." Physiol Behav **39**(4): 477-481.

Axelrod, J. (1971). "Noradrenaline: fate and control of its biosynthesis." Science **173**(3997): 598-606.

Azar, C., M. Valentine, J. Trausch-Azar, T. Druley, D. M. Nelson and A. L. Schwartz (2018). "RNA-Seq identifies genes whose proteins are transformative in the differentiation of cytotrophoblast to syncytiotrophoblast, in human primary villous and BeWo trophoblasts." Sci Rep **8**(1): 5142.

Balkovetz, D. F., C. Tiruppathi, F. H. Leibach, V. B. Mahesh and V. Ganapathy (1989). "Evidence for an imipramine-sensitive serotonin transporter in human placental brush-border membranes." J Biol Chem **264**(4): 2195-2198.

Bangma, J. T., H. Hartwell, H. P. Santos, T. M. O'Shea and R. C. Fry (2021). "Placental programming, perinatal inflammation, and neurodevelopment impairment among those born extremely preterm." Pediatric Research **89**(2): 326-335.

Barker, D. J., A. R. Bull, C. Osmond and S. J. Simmonds (1990). "Fetal and placental size and risk of hypertension in adult life." BMJ (Clinical research ed.) **301**(6746): 259-262.

Barker, D. J. P., K. M. Godfrey, P. D. Gluckman, J. E. Harding, J. A. Owens and J. S. Robinson (1993). "Fetal nutrition and cardiovascular disease in adult life." The Lancet **341**(8850): 938-941.

Belisle, S., A. Petit, N. Gallo-Payet, J.-G. Lehoux, D. Bellabarba, E. Escher and G. Guillon (1992). "Endocrine control of hPL and hCG production by the human placenta." Placenta **13**: 163-172.

Ben-Jonathan, N. and R. E. Maxson (1978). "ELEVATION OF DOPAMINE IN FETAL PLASMA AND THE AMNIOTIC FLUID DURING GESTATION*†." Endocrinology **102**(2): 649-652.

Berke, J. D. (2018). "What does dopamine mean?" Nature neuroscience **21**(6): 787-793.

Boden, S. R. and A. Wesley Burks (2011). "Anaphylaxis: a history with emphasis on food allergy." Immunological reviews **242**(1): 247-257.

Bonnin, A., N. Goeden, K. Chen, M. L. Wilson, J. King, J. C. Shih, R. D. Blakely, E. S. Deneris and P. Levitt (2011). "A transient placental source of serotonin for the fetal forebrain." Nature **472**(7343): 347-350.

Bonnin, A. and P. Levitt (2011). "Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain." Neuroscience **197**: 1-7.

Bzoskie, L., L. Blount, K. Kashiwai, Y. T. Tseng, W. W. Hay, Jr. and J. F. Padbury (1995). "Placental norepinephrine clearance: in vivo measurement and physiological role." Am J Physiol **269**(1 Pt 1): E145-149.

Costa, M. A. (2016). "The endocrine function of human placenta: an overview." Reprod Biomed Online **32**(1): 14-43.

Cote, F., C. Fligny, E. Bayard, J. M. Launay, M. D. Gershon, J. Mallet and G. Vodjdani (2007). "Maternal serotonin is crucial for murine embryonic development." Proc Natl Acad Sci U S A **104**(1): 329-334.

Coyle, J. T. and J. Axelrod (1972). "Tyrosine hydroxylase in rat brain: developmental characteristics." J Neurochem **19**(4): 1117-1123.

De Felice, L. (2004). "An overview of monoamine transporters: mechanism, structure and function." Fundamental & Clinical Pharmacology **18**: 132-132.

Ebert, S. N. and D. G. Taylor (2006). "Catecholamines and development of cardiac pacemaking: an intrinsically intimate relationship." Cardiovasc Res **72**(3): 364-374.

Eisenhofer, G., A. Aneman, P. Friberg, D. Hooper, L. Fåndriks, H. Lonroth, B. Hunyady and E. Mezey (1997). "Substantial production of dopamine in the human gastrointestinal tract." The Journal of clinical endocrinology and metabolism **82**(11): 3864-3871.

Elad, D., R. Levkovitz, A. J. Jaffa, G. Desoye and M. Hod (2014). "Have We Neglected the Role of Fetal Endothelium in Transplacental Transport?" Traffic **15**(1): 122-126.

Elmetwally, M. A., Y. Lenis, W. Tang, G. Wu and F. W. Bazer (2018). "Effects of catecholamines on secretion of interferon tau and expression of genes for synthesis of polyamines and apoptosis by ovine trophectoderm." Biol Reprod **99**(3): 611-628.

Eyles, D., J. Feldon and U. Meyer (2012). "Schizophrenia: do all roads lead to dopamine or is this where they start? Evidence from two epidemiologically informed developmental rodent models." Transl Psychiatry **2**(2): e81.

Fraser-Spears, R., A. M. Krause-Heuer, M. Basiouny, F. P. Mayer, R. Manishimwe, N. A. Wyatt, J. C. Dobrowolski, M. P. Roberts, I. Greguric, N. Kumar, W. Koek, H. H. Sitte, P. D. Callaghan, B. H. Fraser and L. C. Daws (2019). "Comparative analysis of novel decynium-22 analogs to inhibit transport by the low-affinity, high-capacity monoamine transporters, organic cation transporters 2 and 3, and plasma membrane monoamine transporter." Eur J Pharmacol **842**: 351-364.

Ganapathy, V., S. Ramamoorthy and F. H. Leibach (1993). "Transport and metabolism of monoamines in the human placenta: A review." Placenta **14**: 35-51.

Garabal, M. V., R. M. Arévalo, M. D. Díaz-Palarea, R. Castro and M. Rodríguez (1988). "Tyrosine availability and brain noradrenaline synthesis in the fetus: control by maternal tyrosine ingestion." Brain Research **457**(2): 330-337.

Gennser, G. and W. Von Studnitz (1975). "Noradrenaline synthesis in human fetal heart." Experientia **31**(12): 1422-1424.

Goeden, N., J. C. Velasquez and A. Bonnin (2013). "Placental tryptophan metabolism as a potential novel pathway for the developmental origins of mental diseases." Translational Developmental Psychiatry **1**(1): 20593.

Hansson, S. R., B. Bottalico, V. Noskova and B. Casslén (2009). "Monoamine transporters in human endometrium and decidua." Human Reproduction Update **15**(2): 249-260.

Herlenius, E. and H. Lagercrantz (2001). "Neurotransmitters and neuromodulators during early human development." Early Human Development **65**(1): 21-37.

Horackova, H., R. Karahoda, L. Cerveny, V. Vachalova, R. Ebner, C. Abad and F. Staud (2021). "Effect of Selected Antidepressants on Placental Homeostasis of Serotonin: Maternal and Fetal Perspectives." Pharmaceutics **13**(8): 1306.

Chatuphonprasert, W., K. Jarukamjorn and I. Ellinger (2018). "Physiology and Pathophysiology of Steroid Biosynthesis, Transport and Metabolism in the Human Placenta." Frontiers in pharmacology **9**: 1027-1027.

Chua, B. A. and A. M. Perks (1998). "The effect of dopamine on lung liquid production by in vitro lungs from fetal guinea-pigs." J Physiol **513 (Pt 1)**(Pt 1): 283-294.

Illsley, N. P. (2000). "Glucose transporters in the human placenta." Placenta **21**(1): 14-22.

Jansson, T. (2001). "Amino acid transporters in the human placenta." Pediatr Res **49**(2): 141-147.

Jiraskova, L., L. Cerveny, S. Karbanova, Z. Ptackova and F. Staud (2018). "Expression of Concentrative Nucleoside Transporters (SLC28A) in the Human Placenta: Effects of Gestation Age and Prototype Differentiation-Affecting Agents." Mol Pharm **15**(7): 2732-2741.

Kallol, S., R. Moser-Haessig, C. E. Ontsouka and C. Albrecht (2018). "Comparative expression patterns of selected membrane transporters in differentiated BeWo and human primary trophoblast cells." Placenta **72-73**: 48-52.

Karahoda, R., C. Abad, H. Horackova, P. Kastner, J. Zaugg, L. Cerveny, R. Kucera, C. Albrecht and F. Staud (2020). "Dynamics of Tryptophan Metabolic Pathways in Human Placenta and Placental-Derived Cells: Effect of Gestation Age and Trophoblast Differentiation." Frontiers in Cell and Developmental Biology **8**(937).

Karahoda, R., H. Horackova, P. Kastner, A. Matthios, L. Cerveny, R. Kucera, M. Kacerovsky, J. Duintjer Tebbens, A. Bonnin, C. Abad and F. Staud (2020). "Serotonin homeostasis in the materno-foetal interface at term: Role of transporters (SERT/SLC6A4 and OCT3/SLC22A3) and monoamine oxidase A (MAO-A) in uptake and degradation of serotonin by human and rat term placenta." Acta Physiologica **229**(4): e13478.

Kim, H. J., P. O. Koh, S. S. Kang, W. Y. Paik and W. S. Choi (2001). "The localization of dopamine D2 receptor mRNA in the human placenta and the anti-angiogenic effect of apomorphine in the chorioallantoic membrane." Life Sci **68**(9): 1031-1040.

Kim, M. O., J. H. Kim, W. S. Choi, B. H. Lee, G. J. Cho, S. M. Roh, B. J. Lee, S. G. Kang, C. H. Kim and S. H. Baik (1997). "Colocalization of dopamine D1 and D2 receptor mRNAs in rat placenta." Mol Cells **7**(6): 710-714.

Knofler, M., S. Haider, L. Saleh, J. Pollheimer, T. Gamage and J. James (2019). "Human placenta and trophoblast development: key molecular mechanisms and model systems." Cell Mol Life Sci **76**(18): 3479-3496.

Kratimenos, P. and A. A. Penn (2019). "Placental programming of neuropsychiatric disease." Pediatric Research **86**(2): 157-164.

Kurosawa, A., H. Kageyama, T. M. John, R. Hirota and S. Itoh (1980). "Effect of Neonatal Hydrocortisone Treatment on Brain Monoamines in Developing Rats." Japanese Journal of Pharmacology **30**(2): 213-220.

Lager, S. and T. L. Powell (2012). "Regulation of Nutrient Transport across the Placenta." Journal of Pregnancy **2012**: 179827.

Lanoix, D., H. Beghdadi, J. Lafond and C. Vaillancourt (2008). "Human placental trophoblasts synthesize melatonin and express its receptors." J Pineal Res **45**(1): 50-60.

Laurent, L., K. Deroy, J. St-Pierre, F. Cote, J. T. Sanderson and C. Vaillancourt (2017). "Human placenta expresses both peripheral and neuronal isoform of tryptophan hydroxylase." Biochimie **140**: 159-165.

Lee, N., M. F. Hebert, B. Prasad, T. R. Easterling, E. J. Kelly, J. D. Unadkat and J. Wang (2013). "Effect of gestational age on mRNA and protein expression of polyspecific organic cation transporters during pregnancy." Drug metabolism and disposition: the biological fate of chemicals **41**(12): 2225-2232.

Liu, C. and P. S. Kaeser (2019). "Mechanisms and regulation of dopamine release." Current opinion in neurobiology **57**: 46-53.

Manyonda, I. T., D. M. Slater, C. Fenske, D. Hole, M. Y. Choy and C. Wilson (1998). "A role for noradrenaline in pre-eclampsia: towards a unifying hypothesis for the pathophysiology." Br J Obstet Gynaecol **105**(6): 641-648.

Meyer zu Schwabedissen, H. E., G. Jedlitschky, M. Gratz, S. Haenisch, K. Linnemann, C. Fusch, I. Cascorbi and H. K. Kroemer (2005). "Variable expression of MRP2 (ABCC2) in human placenta: influence of gestational age and cellular differentiation." Drug Metab Dispos **33**(7): 896-904.

Moore, J. J., Jr. and J. A. Whitsett (1982). "The beta-adrenergic receptor in mammalian placenta: species differences and ontogeny." Placenta **3**(3): 257-268.

Murrin, L. C., J. D. Sanders and D. B. Bylund (2007). "Comparison of the maturation of the adrenergic and serotonergic neurotransmitter systems in the brain: implications for differential drug effects on juveniles and adults." Biochem Pharmacol **73**(8): 1225-1236.

Nagatsu, T. (2007). "The catecholamine system in health and disease -Relation to tyrosine 3-monooxygenase and other catecholamine-synthesizing enzymes." Proceedings of the Japan Academy. Series B, Physical and biological sciences **82**(10): 388-415.

Napso, T., H. E. J. Yong, J. Lopez-Tello and A. N. Sferruzzi-Perri (2018). "The Role of Placental Hormones in Mediating Maternal Adaptations to Support Pregnancy and Lactation." Frontiers in Physiology **9**(1091).

Nguyen, T. T., Y. T. Tseng, B. McGonnigal, J. P. Stabila, L. A. Worrell, S. Saha and J. F. Padbury (1999). "Placental biogenic amine transporters: in vivo function, regulation and pathobiological significance." Placenta **20**(1): 3-11.

Novotna, M., A. Libra, M. Kopecky, P. Pavek, Z. Fendrich, V. Semecky and F. Staud (2004). "P-glycoprotein expression and distribution in the rat placenta during pregnancy." Reprod Toxicol **18**(6): 785-792.

O'Donnell, K. J. and M. J. Meaney (2016). "Fetal Origins of Mental Health: The Developmental Origins of Health and Disease Hypothesis." American Journal of Psychiatry **174**(4): 319-328.

Ohtani, N., T. Goto, C. Waeber and P. G. Bhide (2003). "Dopamine modulates cell cycle in the lateral ganglionic eminence." The Journal of neuroscience : the official journal of the Society for Neuroscience **23**(7): 2840-2850.

Padbury, J. F. (1989). "5 - Functional maturation of the adrenal medulla and peripheral sympathetic nervous system." Baillière's Clinical Endocrinology and Metabolism **3**(3): 689-705.

Paravati, S., A. Rosani and S. J. Warrington (2022). Physiology, Catecholamines. StatPearls. Treasure Island (FL), StatPearls Publishing

Peleg, D., R. A. Munsick, D. Diker, J. A. Goldman and N. Ben-Jonathan (1986). "Distribution of catecholamines between fetal and maternal compartments during human pregnancy with emphasis on L-dopa and dopamine." J Clin Endocrinol Metab **62**(5): 911-914.

Popolo, M., D. M. McCarthy and P. G. Bhide (2004). "Influence of dopamine on precursor cell proliferation and differentiation in the embryonic mouse telencephalon." Developmental neuroscience **26**(2-4): 229-244.

Previc, F. H. (2007). "Prenatal influences on brain dopamine and their relevance to the rising incidence of autism." Med Hypotheses **68**(1): 46-60.

Ramamoorthy, S., F. H. Leibach, V. B. Mahesh and V. Ganapathy (1992). "Active transport of dopamine in human placental brush-border membrane vesicles." Am J Physiol **262**(5 Pt 1): C1189-1196.

Ramamoorthy, S., P. D. Prasad, P. Kulanthaivel, F. H. Leibach, R. D. Blakely and V. Ganapathy (1993). "Expression of a cocaine-sensitive norepinephrine transporter in the human placental syncytiotrophoblast." Biochemistry **32**(5): 1346-1353.

Robinson, P. D., C. K. Schutz, F. Macciardi, B. N. White and J. J. Holden (2001). "Genetically determined low maternal serum dopamine beta-hydroxylase levels and the etiology of autism spectrum disorders." Am J Med Genet **100**(1): 30-36.

Rosenfeld, C. S. (2021). "The placenta-brain-axis." Journal of neuroscience research **99**(1): 271-283.

Rothbauer, M., N. Patel, H. Gondola, M. Siwetz, B. Huppertz and P. Ertl (2017). "A comparative study of five physiological key parameters between four different human trophoblast-derived cell lines." Scientific Reports **7**(1): 5892.

Ruohonen, S. (2022). "TRANSGENIC MICE OVEREXPRESSING NEUROPEPTIDE Y: AN EXPERIMENTAL MODEL OF METABOLIC AND CARDIOVASCULAR DISEASES."

Saboory, E., M. Ghasemi and N. Mehranfard (2020). "Norepinephrine, neurodevelopment and behavior." Neurochem Int **135**: 104706.

Sagrillo-Fagundes, L., E. M. Assunção Salustiano, R. Ruano, R. P. Markus and C. Vaillancourt (2018). "Melatonin modulates autophagy and inflammation protecting human placental trophoblast from hypoxia/reoxygenation." Journal of Pineal Research **65**(4): e12520.

Sferruzzi-Perri, A. N. and E. J. Camm (2016). "The Programming Power of the Placenta." Frontiers in Physiology **7**(33).

Schulz, C., G. Eisenhofer and H. Lehnert (2004). "Principles of catecholamine biosynthesis, metabolism and release." Front Horm Res **31**: 1-25.

Siaterli, M. Z., D. Vassilacopoulou and E. G. Fragoulis (2003). "Cloning and expression of human placental L-Dopa decarboxylase." Neurochem Res **28**(6): 797-803.

Sodha, R. J., M. Proegler and H. Schneider (1984). "Transfer and metabolism of norepinephrine studied from maternal-to-fetal and fetal-to-maternal sides in the in vitro perfused human placental lobe." Am J Obstet Gynecol **148**(4): 474-481.

Staud, F. and M. Ceckova (2015). "Regulation of drug transporter expression and function in the placenta." Expert Opin Drug Metab Toxicol **11**(4): 533-555.

Staud, F. and R. Karahoda (2018). "Trophoblast: The central unit of fetal growth, protection and programming." Int J Biochem Cell Biol **105**: 35-40.

Sundström, E., S. Kölare, F. Souverbic, E. B. Samuelsson, H. Pschera, N. O. Lunell and Å. Seiger (1993). "Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester." Developmental Brain Research **75**(1): 1-12.

Thomas, S. A., A. M. Matsumoto and R. D. Palmiter (1995). "Noradrenaline is essential for mouse fetal development." Nature **374**(6523): 643-646.

Torres, G. E., R. R. Gainetdinov and M. G. Caron (2003). "Plasma membrane monoamine transporters: Structure, regulation and function." Nature Reviews Neuroscience **4**(1): 13-25.

Vähäkangas, K. and P. Myllynen (2009). "Drug transporters in the human blood-placental barrier." British Journal of Pharmacology **158**(3): 665-678.

Vieira-Rocha, M. S., P. Rodríguez-Rodríguez, J. B. Sousa, M. C. González, S. M. Arribas, A. L. López de Pablo and C. Diniz (2019). "Vascular angiotensin AT1 receptor neuromodulation in fetal programming of hypertension." Vascul Pharmacol **117**: 27-34.

Walters, D. V. and R. E. Olver (1978). "The Role of Catecholamines in Lung Liquid Absorption at Birth." Pediatric Research **12**(3): 239-242.

Whiting, M. J. and M. P. Doogue (2009). "Advances in biochemical screening for phaeochromocytoma using biogenic amines." Clin Biochem Rev **30**(1): 3-17.

Zhu, Y., W. Zhang, M. Chen, N. Liu and J. Guo (2002). "[Study on expression of norepinephrine and dopamine placental tissues of normal pregnancy and pregnancy induced hypertension syndrome]." Zhonghua Fu Chan Ke Za Zhi **37**(3): 142-145.