Chapter 3

Role of Nitric Oxide Synthase in Normal Brain Function and Pathophysiology of Neural Diseases

Melih Dagdeviren

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67267

Abstract

Nitric oxide synthase has three isoforms; according to their roles and tissues or cells they are involved. Neuronal NOS (nNOS) takes place in neuronal signalling, endothelial NOS (eNOS) takes place in vasodilation and inducible NOS (iNOS) takes place in immune responses. nNOS and eNOS are dominant but all isoforms have various roles in the central nervous system. nNOS and eNOS separately or together works in healthy brain during cognitive processes and in unhealthy brain during the pathology of related diseases. These roles were shown by inhibitor applied or by transgenic animal model studies and also by investigating the diseases at the molecular level. Besides, it is possible to say that iNOS has roles in some neurological pathologies creating immune responses. Three different isoforms mainly serve in different systems so there are lots of researchers from various disciplines working collaterally not knowing the others related works about NOSs. Because of this, a comprehensive chapter will be valuable for neuroscientists working with either healthy or unhealthy brains. The purpose of this chapter is to gather an overview of NOSs duties during the normal processes of the brain like learning and memory formation and abnormal processes such as depression, schizophrenia and brain cancers.

Keywords: NOS, learning, depression, brain cancers

1. Introduction

Nitric oxide (NO) also known as nitrogen oxide or nitrogen monoxide is a small molecule, which is a gaseous secondary messenger in mammalian cells [1]. Since the early 1990s, the importance of that molecule for biological systems has been investigated by various branches of related fields. Robert F. Furchgott, Louis J. Ignarro and Farid Murad earned a Nobel Prize in physiology or medicine in the year 1998 about the findings of the signalling properties of NO in cardiovascular systems [2]. Thus, the importance of that molecule for biological systems was emphasized.

The pathways that create NO in an organism can differ from system to system and tissue to tissue. Inside the mammalian cells NO is produced as a co-product of a biochemical activity catalysed by the nitric oxide synthase (NOS) enzymes. NOS enzymes are flavoenzymes that contain iron-heme, and these enzymes need nicotinamide-adenine-dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and (6R-)5, 6, 7, 8-tetrahydro-L-biopterin (BH_4) as cofactors to convert substrate L-arginine to L-citrulline. During that reaction NO is formed. As a water-soluble gas, NO can easily diffuse to neighbouring cells; however, the diffusion is limited because of the short half-life of NO [3].

NOS enzyme has three isoforms, two of them are constitutive isoforms: endothelial NOS (eNOS/NOS3) essentially found in the vascular system and neuronal NOS (nNOS/NOS1) essentially found in the nervous system. The third isoform is inducible isoform: inducible NOS (iNOS/NOS2) principally found in immune system cells [4]. NOSs are homodimeric enzymes and for NO producing reactions NOSs transfer electrons from NADPH to heme via FAD and FMN in the amino terminal. The oxygenase domain of the enzyme also binds the BH_4, O_2 and L-arginine. L-arginine is oxidized to L-citrullin and NO. All three isoforms bind calmodulin, which work as a molecular switch for NOSs [5].

eNOS is one of the constitutive isoforms, which is generally present in the vascular endothelial cells. eNOS is dominantly expressed by endothelial cells and expressed in little amounts by some other cardiovascular system cells such as cardiomyocytes, erythrocytes, leucocytes, platelets and microparticles in the blood. Ca^{2+} concentration is an important factor for eNOS activation, also haemodynamic forces, hypoxia, catecholamines, exercise, G-protein activation and post-translational modifications activate eNOS. To response these various stimulants, eNOSs sometimes gather in special sections inside cells, these special sections have caveolin-binding activity. These various stimulant response adaptations make eNOS differ from the other isoforms. Because the other two isoforms are regulated by a smaller number of factors [6], NO is very important to keep the vascular homeostasis at a balance. NO synthesized freshly, travels to the neighbouring cells. Then in the vessel walls, inside the cells, NO binds to guanylate cyclase (GC), thus cyclic guanosine monophosphate (cGMP) concentration increase, Ca^{2+} channels start to open and when calcium inflows the smooth muscle relaxation is triggered [7].

The three isoforms have small differences between each other. nNOS was first identified in brain and have the biggest molecular weight than the other isoforms with 160 kDa, and eNOS and iNOS having 133 and 131 kDa, respectively. The difference is caused by PDZ domain of nNOS, and caveolin-binding site of eNOS [8]. nNOS is mainly expressed in nervous system cells and is very important for neural functions. The primary nNOS expressing cells are neurons in the brain and also neurons of hypothalamus, pineal gland, spinal cord and nerves innervating other organs. Besides nNOS is also expressed in myocytes, epithelial cells, macula densa cells, testis-urethra cells, mast cells and neutrophils of various mammals [9]. nNOS is also Ca^{2+}-calmodulin dependent, and the activation of nNOS is also regulated by the phosphorylation and neurotransmitter activity. N-methyl-D-aspartate (NMDA) receptors have the key role for nNOS activation in neurons. Sometimes nNOS activation is triggered by

http://dx.doi.org/10.5772/67267

presynaptic neurons; glutamate released from the presynaptic neuron increases the Ca^{2+} in the post-synaptic neuron through NMDA receptor, which increased Ca^{2+} activating the nNOS in the post-synaptic neuron. Then nNOS produce NO, which diffuses back to the presynaptic neuron and triggers soluble GC [4, 10].

There are several answers to the question why NO is important for the brain. That freely diffusible gaseous secondary messenger can be a lifesaver or villain for neurological processes [11]. Thus, nNOS became as important as NO in the brain. nNOS plays an important role in neuronal function, memory formation, sexually different behaviours, neurological regulations and for therapeutics.

nNOS in the whole brain and additionally eNOS in the hippocampus and somatosensory cortex are involved in NO and cGMP formation. These secondary messengers activate synaptic plasticity in the hippocampus, cerebral cortex, amygdala, striatum and cerebellum [12]. In several murine, nNOS inhibition studies and an nNOS knockout mice study revealed that it is crucial for memory formation and recognition of memory [13].

There are lots of studies that try to explain the link between nNOS and behaviour at the molecular level. Sex difference is a very important variable for behavioural studies. There is a significant difference between male and female mice according to their aggressive behaviour if their nNOS gene were knocked-out. Besides in a NOS inhibition study, it was shown that male rats tended to show female-like perceptual style behaviour [14, 15]. It is possible to say that NO and nNOS are important molecules for sex difference dependent behavioural studies.

There are lots of inhibitors for NOSs; arginine-like chemicals bind NOSs instead of L-arginine to inhibit the reaction catalysed by NOSs. Most widely used inhibitors are nitro-L-arginine (L-NA), nitro-L-arginine methyl ester (L-NAME) and N-monomethyl L-arginine (L-NMMA). These inhibitors are not selective for a specific isoform, but there are also selective inhibitors available. The non-selective inhibitors are important as selective ones. These non-selective inhibitors helped to suppress both eNOS and nNOS, because both isoforms take place in regulating vascular activities in the brain. With that inhibitors it was shown that NO/NOS pathway is very important for regulating the cerebral blood flow within the healthy brain, and also very important for the ischaemic brain. The studies done with the ischaemic brain have revealed that NO is also a double-edged sword for that pathology. Scientists trying to find a treatment for the ischaemic brain over NO pathway should consider the risk of manipulating that pathway [16].

There have been some other studies on NO/nNOS pathway for therapeutic concerns in the ischaemic brain. Inhibiting or activating nNOS is a point of view where scientists are dealing with the internal sources of the organism. However, there is another option: to supply NO from outside of the organism. There are some studies that supply NO to the organism by inhalation to treat injured brains. According to the results of these studies, the endogenous sources are generally a better target to manipulate. But for the ischaemic brain, the external NO supply is useful at the ischaemia phase, not in the reperfusion phase. It is possible to interpret

that it is not easy to separate the ischaemic and reperfusion phases if it is not a controlled operation. Therefore, external NO inhalation seems not efficient for ischaemic conditions [17].

There are lots of therapeutic strategies to overcome different pathologies involving NOSs. But before developing a technique or a new technology for treatment, the molecular mechanisms behind the normal conditions and/or pathological conditions must be exhibited thoroughly to avoid the devastating side effects of the potential therapy.

The only inducible isoform iNOS is not readily expressed inside the cells. This isoform is mostly expressed in macrophages when there is a pathogenic condition for host defence. There are some differences between constitutive and inducible forms. iNOSs are not Ca^{2+} dependent, also when induced they produce NO continuously, they are affected by glucocorticoids and less labile. The action of iNOS enzyme starts with binding of cytokines and/or lipopolysaccharides to cell surface receptors instead of calcium influx. Then the similar reaction occurs to produce NO. For stopping the activity, glucocorticoids bind the secondary messengers of cytokine-triggered cascade [18]. The stimuli activating the mice iNOS are not activating the human iNOS [19]. This situation should mislead the researchers while developing a therapeutic strategy. It is more serious if the strategy will be depending on only the animal experiment results.

2. Roles of nitric oxide synthase enzymes in healthy brain

It is important to put forth the molecular mechanism of a molecule for normal conditions. Under this heading, it was aimed to introduce and discuss the roles of NOS enzymes in the healthy brain functions. NOS enzymes have several roles in the healthy brain; especially NO is crucial for learning and memory formation. Besides, NOS enzymes have roles in retinal function, hearing and molecular mechanisms in the cochlea.

2.1. Roles of NOSs in learning and memory formation

One of the main duties of NOS enzymes in the healthy brain is learning and memory formation. Learning is a complex behaviour but the molecular mechanisms of memory formation are mostly enlightened. During the second half of the 1990s, scientists proposed that NO has a role in learning and memory and in neuronal plasticity. Experiments were designed to look for the role of NO/NOSs in learning. NO has a part in both long-term potentiations (LTP) in the hippocampus and long-term depression (LTD) in cerebellum, which are basic mechanisms for memory formation [20].

Neuron-neuron interaction isles called synapses are very plastic; LTP is a form of that plasticity. LTP occur due to the repetitive stimulation of the presynaptic neuron. A consequence of that repetitive stimulation is the influx of calcium to post-synaptic neuron via NMDA receptor, which in turn increases the Ca^{2+} concentration in the cell. For completing the circuit of LTP, there should be a retrograde messenger. Studies done with knockout mice and inhibitors, revealed that NO is that retrograde messenger for LTP in the hippocampus. But both nNOS and eNOS take place to from LTP [21]. Selective inhibitors for nNOS also showed that there is a

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deficiency in memory formation in various vertebrates [22]. Not only NO/NOS pathway studies showed the initiation of NO in LTP, but also studies looking for the sGC activity in rat hippocampus support the findings of NO/NOS and LTP correlation [23]. LTD occurs in cerebellar purkinje cells. There are two cells docking each other, climbing fibres and purkinje cells. Also, interneurons are neighbouring that synapse. NO released from interneurons and diffuse to the purkinje cells, then sGC initiates in LTD formation with similar action of LTP but in opposite direction [24, 25].

For memory formation by NOS there is also other signalling molecules in the cascade, like extracellular signal-regulated kinase (ERK). Thus, memory formation processes become more complex [26]. In the stress-exposed rats' hippocampi nNOS activity was diminished. This stress causes a deficit in learning and memory processes in these stressed animals. Same learning and memory problems have seen in hypoxic/ischaemic hippocampi of rats [27].

Within memory-forming circuits between neurons, NO can act as a volume transmitter. Thus, that small molecule can affect the remote parts of the brain at the same time. Also for conceptual learning studies done with invertebrates, it is revealed that indirectly NO/NOS mechanisms take place in learning [28]. It was shown that NOS inhibitors hinder motor learning in adult animals, and the formation of olfactory memories. The motor learning malfunction may arise from the non-selective inhibitors [29, 30]. These are some results explaining the roles of NO/NOS pathway in memory formation.

2.2. Roles of NOSs in seeing and retinal function

Retinal function is very important for developed organisms, and retinal function has various molecular pathways. Also, NO/NOS mechanism is one of the regulators of action of seeing and functioning of the retina. Seeing is a complex process that takes place in visual cortex but the retinal function is essential to carry the visual signals to that processor area of the brain.

In the retina, NOS is found in retinal neurons, pigment epithelium, amacrine and ganglion cells, nerve fibres in the outer and inner plexiform layers and in photoreceptor ellipsoids. But in normal rats' optic nerve, there is not any NOS enzyme present [31]. In photoreceptor cells, ion channels activate the reaction. In the inner segment, Ca^{2+} concentration increases, then activate the nNOS and thus NO produced. That NO activates the sGC in the same cell not inside the other cells. Then GC pathway will be triggered in on-bipolar cells. Besides in amacrine cell-ganglion cell-bipolar cell synapse; NO produced in amacrine cell, diffuse to the ganglion cell and activate the GC and this cause depolarization [31, 32]. Depolarization in the retina is an essential step for seeing.

There are some studies ranging from developmental biology to animal behaviour that explored the roles of NO/NOS pathway in the retinal function. In the developing human foetal eye nNOS and eNOS are expressed at the same time but in different compartments. Besides, nuclear nNOS was found in progenitor cells, endothelial cells and pericytes. Because of this situation, nNOS may have a transcription regulatory role for some cells during ocular vasculogenesis and angiogenesis [33]. In a study an inhibitor was used which selectively triggered photoreceptor cell death to determine the role of NO in retinal degeneration.

Scientists found that there is a correlation between the increasing NO levels and nNOS activity and mouse retinal cell death [34]. Chick retinas removed during a study revealed the role of NO/NOS mechanism in the brain. The group looked for the expression of NOSs in visual structures of the brain. The NOSs expressions increased after retinal removal. This shows that the NO/NOS mechanism has a role in plasticity processes in visual parts of the chick brain [35].

To unveil the role of NO in optic nerve head blood flow, scientists conducted a NOS inhibitor study in healthy humans. Subjects received L-NMMA and performed isometric exercise during the study. They proposed that NO has an important role in basal optic nerve head blood flow but not in autoregulatory response induced by exercise [36].

In cultured retinal neurons, NO inhibit apoptosis via activating various kinases. In cultured chick embryonic retinal neurons, both endogenous and exogenous NO promoted AKT signalling pathway and probably survival mechanisms [37]. In goldfish optic nerve, scientists showed that NO signalling pathway through nNOS activation has a crucial role in nerve regeneration [38].

In a knockout mice study, roles of all three isoforms were investigated comparatively. It was shown that not having one of the three isoforms did not alter the intraocular pressure or number of neurons in the eye. But eNOS is crucial for endothelium-dependent dilation of murine eye arteries. In conclusion, NOSs are involved in the regulation of ocular vascular tone and blood flow [39].

2.3. Roles of NOSs in hearing and cochlea

Hearing starts at the outer ear and stimuli travels through tympanum and bones and finally arrives at the cochlea, where hair cells and nerve fibres take action. NO and NOSs have roles in hearing function and cochlear activities. And hearing process happens in the auditory cortex.

NO act as a neurotransmitter and/or neuromodulator in the cochlea [40, 41]. In recent years, it was revealed that NO has also a potassium channel modulator role in inner hair cells. Therefore, NO-potassium modulation may be responsible for high-frequency hearing impairment [42].

NO/cGMP pathway was triggered by nerve fibres innervating outer hair cells, NO was produced in these cells and released. But NO affects Deiters' cells and Heusen's cells and not the outer hair cells. Also, nNOS takes place in acoustic overstimulation condition. Inner hair cells release excess glutamate during continuous stimulation. This glutamate increase calcium influx to afferent dendrites where nNOS produce NO. Then overproduction of NO due to acoustic overstimulation kills afferent dendrites because of excitotoxicity [43]. Auditory nerve, lateral wall, vestibule and cochlear neuroepithelium are the areas where NOS activity is the highest in the auditory system. nNOS is the predominant isoform in the cochlea [44].

In a gerbil study, researchers examined the role of NO in cochlelar excitotoxicity. Cochlear compound action potentials thresholds were recorded with NOS inhibitor and glutamate exposed conditions. Overstimulation with glutamate caused NO-mediated excitotoxicity in the cochlea. NOS inhibition should be neuroprotective for cochlea [45].

http://dx.doi.org/10.5772/67267

To trigger iNOS expression in cochlea, bacterial lipopolysaccharides and tumour necrosis factor α was injected to guinea pigs. The iNOS expression was higher than eNOS and nNOS during that experiment. iNOS were localized in the cochlea's blood vessel walls of the spiral ligament and the modiolus, in the organ of Corti, in the limbus, in nerve fibres and in spiral ganglion [46]. This dispersed iNOS is caused by the bacterial lipopolysaccharide-triggered immune response. In another study with immonostaining data, the distribution of NOSs was determined in the auditory system. nNOS was dispersed in the inner and outer hair cells, spiral ganglion cells, cells of the stria vascularis, spiral ligament cells and vessel cells near the modiolus. eNOS was dispersed in vascular endothelial cells, and in spiral ganglion cells. If there were not immune stimulus there would be no iNOS in cochlea [47].

3. Roles of nitric oxide synthase in unhealthy brain

NOS enzymes have important duties during pathophysiology of unhealthy brains. Unlike healthy brains, the roles not only depend on signal transduction, but also on anabolic/catabolic mechanisms. Under that heading various diseases, pathologies, malfunctions and disabilities of brains will be evaluated from the point of view of NOSs.

3.1. Roles of NOSs in neuropsychiatric diseases

It is very hard to diagnose the neuropsychiatric diseases properly; however, there are globally accepted parameters. Although the criteria for diagnosis is generally evaluated at certain times by prestigious committees and there is still hardships for diagnosis. One of the main goals of the scientists working on neuropsychiatric disorders is to pair a marker molecule with a disease to facilitate the diagnosis. So far there is not any coupling for any NOSs for any neuropsychiatric disorder as a marker but NOSs have various roles for these diseases. Anxiety, depression/major depression and tendency for suicide are important and common neuropsychiatric disorders. NOS has roles for these abnormalities.

In patients with depression it was shown that NO expression altered via eNOS. Besides NO modulates neuropeptides, such as vasopressin, oxytocin and corticotrophin-releasing factor. In patients with depression, these neuropeptides' expression levels were altered. According to post-mortem studies on depression patients, NO signalling was impaired in their hypothalami [48].

Major depression disorder (MDD) will be one of the dominant causes of disability by the year 2020. Antidepressants generally decrease NO levels and/or inhibit NOS activity indirectly. Also NO levels and NOS expressions are higher in MDD patients. Besides, with mice studies, it was shown that there is a correlation between NOS mechanism and depression. NOS inhibitors could be researched as a new target for antidepressant strategies [4, 22].

In a population study done in Taiwan with MDD patients in which the potential genetic variations with healthy and MDD subjects according to their nNOS polymorphisms was researched. There is no difference between subjects; the frequencies are similar for controls

and MDD patients [49]. In an autopsy and tissue bank-based study from Holland, there is decrease in nNOS expression in the anterior cingulate cortex of MDD-diagnosed people [50].

In mice along with stress-induced depression, nNOS expression increases in the hippocampi. Due to excitotoxicity neurogenesis in hippocampi is suppressed. To inhibit nNOS signalling may be a novel approach for depression treatment [51]. Also, iNOS is involved in stress-triggered depression. NO derived from iNOS and mRNA levels of iNOS increased in cortices of depression model applied mice [52].

Also in a population study there is no correlation between genetic polymorphisms and MDD. In Japanese population MDD patients were investigated for polymorphisms in their nNOS genes, but found no variation between controls and MDD [53]. From a Czech Republic population genetic study, there is also no correlation between eNOS and MDD [54]. A population study from United Kingdom found a correlation between single nucleotide polymorphisms in nNOS gene and psychosocial stress-triggered depression. The individuals carrying those polymorphisms have a tendency to develop depression if they face financial hardship [55].

There is a link between vascular problems, depressive behaviours and NO metabolism. When vascular dysfunction emerges after depressive symptoms, the characteristics behind it show lack of bioavailable NO. However, H_2O_2 covers up that deficit [56].

There are studies on ethnopharmacological level to find out if there is a potential drug candidate in botanical material. To investigate NO metabolism is one of the target pathways to detect for antidepressant-like and neuroprotective potential. *Aloysia grattissima* has the potential to treat depressive disorders depending on the NO metabolism manipulating properties of its extracts [57].

nNOS genes' functional promoter repeat length variant contains sites for transcription factors that has strong relation with hyperactive and aggressive behaviour. Thus, nNOS depending on population genetics studies combined with behaviour is a potential research area [58].

For anxiety-like and depression-like behaviours, there is a strong evidential pathway, hypothalamic-pituitary-adrenal axis (HPA). Also on the ecotoxicological aspect, the nutrients for newborns and expectant mothers are very important because they fall in the risk group. The bisphenol-A supplied pregnant female rats' male littermates revealed anxiety-like and depression-like behaviours according to their HPA experiment results. Those littermates' hippocampal nNOS activity was higher than the control animals [59]. nNOS knockout mice show abnormal social behaviour, hyperactivity and impaired remote spatial memory [60].

It is important to demonstrate how nitric oxide synthases are affected in the brain by psychotropic drugs. Orally treated rats with several psychotropics were sacrificed and iNOS gene expressions in the brain were detected. Psychotropics including antidepressants and anxiolytics modulate the gene expression of iNOS in rat brain [61].

It is a complex and controversial psychological situation: suicide. This behaviour has a strong genetic background. In a study it was shown that a single nucleotide polymorphism of nNOS gene has a correlation between suicides in Japanese population, especially in males [62].

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Schizophrenia is a complex illness including biochemical, anatomical and genetic aspects of its pathology. In a post-mortem study, scientists showed that some polymorphic variants of nNOS have overexpression patterns in schizophrenic patients' brains [63].

3.2. Roles of NOSs in neurodegenerative diseases

Alois Alzheimer defined the illness during the early 1900s. Alzheimer's disease (AD) is the most common type of dementia. It generally affects the elderly people and is characterized by aggregating senile plaques and/or neurofibrillary tangles in the brain, which leads to progressive neuronal degeneration and death [64]. Deficits in short-term memory formation are characteristic for AD. Short-term memory formation through LTP is dependent on the NO/NOS pathway. NOSs are very important for creating the new trails between neurons. NOSs take place for activating the presynaptic neurons receptors. However, in the pathology of the AD the harmonization created by NOSs between neurons is blocked by plaques [20–22, 64].

In the brain of an AD patient, β-amyloid peptide aggregates in senile plaques and the arginine within the astrocytes accumulates. These are the classical neuropathology of the disease. Arginine-metabolizing enzymes like NOSs and their association with amyloid peptides are important. The correlation of Aβ-peptide fragments with nNOS has been searched with spectrofluorimetry. The interaction of Aβ-peptide with nNOS causes the molecular movement of two critical tryptophan residues in the structure of the enzyme [65].

Purified nNOS was incubated with Aβ-peptide fragments during 96 hours. The kinetics of the interaction was introduced; nNOS was the amyloidogenic catalyst and all Aβ-peptide fragments were inhibited nNOS [66]. Data from cell culture studies, knockout mice studies and behavioural studies showed that eNOS has a crucial role for decelerating the pathology of AD [67]. If patients with AD start to exercise, they start to increase heart rate, cerebral blood flow and then angiogenesis, which includes NO/eNOS pathway, after which neurogenesis and other self-healing mechanisms are activated [68].

James Parkinson described the disease during the 1810s. Besides the characteristic shakes and tremors, there is a huge molecular mechanism behind Parkinson's disease (PD). The disease is diagnosed generally between 50 and 70 years old people. The mechanism behind PD is still unknown. But the dopamine metabolism decreases significantly in PD; also substantia nigra is one of the potential areas of interest to study the disease.

PD is an illness affecting the dopaminergic pathway in the brain. NO inflict the injuries in dopaminergic neurons. There are several evidences from NOS inhibitor studies about this correlation. However, if a cell goes for the cell death pathway, NO accelerates the process [69]. Some neuroinflammatory responses associated with PD are arousing from NO/iNOS activity. Nitration of α-synuclein triggers the protein aggregation, which worsens the pathology. This mechanism is used to mimic the PD in cell culture [70].

Scientists are trying to create a thorough model of the disease for *in vivo* or *in vitro*, however, so far there is not a completely satisfying model. That is caused by the unknown mechanism behind the illness. To mimic PD a group of researchers castrated the male mice. They followed

the NO/iNOS mechanisms to test their model. According to the iNOS results, the castration of young male mice induces PD pathologies [71].

During PD pathology, several reasons cause cell death in substantia nigra neurons and/or dopaminergic neurons. Nutrition and false diet should be a cause of the disease. As a strategy to add an antioxidant-rich nutritive to the diet may be beneficial. Rats were supplied with pomegranate juice after a PD model. The change in the diet by adding pomegranate juice enhanced the iNOS expression in the animal brain [72].

3.3. Roles of NOSs in brain cancers

There are lots of cancer types present in the head and neck area. But in this section, only cancers originating from cells inside the cranium will be discussed and not the metastatic pathologies.

There are lots of studies done with cultured cancer cells from various mammals; however, studies done with tumour samples from human are rare. Instead of discussing the cell culture studies, it was important to gather the knowledge, which was hard to reach. Most of cancer cell culture studies are done without the healthy control cell lines or experimental models. But some cancer cell culture studies will be discussed.

A biopsy study done with gliomas, and also with meningiomas, showed that all three NOS isoforms were present in the aforementioned tumours. nNOS was significantly dominant in glial cells of gliomas. However, that NOS dense status becomes sparse in the peritumoral tissues [73]. NOSs of tumour cells, opposite to the healthy cells, synthesize predominantly superoxide and peroxynitrite, which generate oxidative stress [74].

Neuroblastoma cell lines are generally used due to their ability to differentiate neuron-like cells. NOS inhibition in Neuro2a cells blocked that differentiation; it is possible to speculate that nNOS may have important roles for dissolving a neuroblastoma tumour [75].

Astrocytomas/gliomas are the origins of cancers from supporting cells of the nervous system. These types of cancers are dominant and dangerous when compared to other brain cancers. In a human biopsy study, it was shown that iNOS has a role in angiogenesis via vascular endothelial growth factor (VEGF), and there is a correlation with iNOS and VEGF for astrocytomas/gliomas but not for reactive astrogliosis samples [76]. Similar results were reported also from a similar study. iNOS expression was increased in grade I, II and III astrocytic gliomas. However, in the same study it was shown that the iNOS expression was decreased for grade IV astrocytic gliomas [77]. In a study with primary astrocytoma biopsy samples prove that eNOS and VEGF work cooperatively in tumour angiogenesis [78]. Besides, in another study, it was shown that astrocytic tumour vessels have more eNOS expression than normal vessels [79]. Also, it is known that nNOS expression increases in glioma tumours.

Craniopharyngiomas consist of the 2–5% of intracranial tumours. In a study done on rats searched for the immune responses of oily cyst content of human craniopharyngioma. Immunohistochemical studies after injection revealed that eNOS expression increased in a time course manner [80].

Medulloblastomas are highly malignant brain tumours generally affecting children and adolescents. In a study done with medulloblastoma cells revealed that NOS has important roles in medulloblastoma cell death. Scientists applied various chemotherapy agents and PDE5 inhibitors to kill cells. Then, they co-treated cells with L-NAME and found out that NOS inhibition accompanying PDE5 inhibitors suppressed cell killing. Most probably NO/NOS has a role in killing of medulloblastoma cells [81]. A study done in knockout mice exhibited that iNOS has an important role in medulloblastoma formation. *Ptch1* heterozygous and iNOS-deficient mice developed medulloblastoma two times higher than *Ptch1* heterozygous and iNOS producing mice. This situation may depend on the granule cell precursors' migration [82].

According to studies done with human ptiutary tumour biopsy samples, scientists tried to reveal the roles of NOSs with the disease. In human pituitary adenomas, eNOS expression increased, whereas iNOS and nNOS were stabile [83]. Another human biopsy study showed that highly invasive adenomas have higher upregulated iNOS, whereas noninvasive adenomas did not have upregulated iNOS. Also, eNOS had upregulation with haemorrhagic adenomas [84].

Schwannomas are benign tumours originating from the Schwann cells. It can arise in any peripheral nerve; however, the frequent version arose around the acoustic nerve. Immunohistochemical study done on human biopsy samples revealed that iNOS has a strong expression for this illness. iNOS was stained around the hyalinized vessels' infiltrating leukocytes in Antoni B areas [85].

In conclusion, it is possible to suggest that both clinical and experimental studies are important on the aspect of NOS and brain cancers. It is very hard to mimic pathologies of some brain cancers both in animals and *in vitro*. Likewise, collecting clinical samples from patients is very hard. NO/NOS pathway is important for brain cancers and more studies needed to reveal the therapeutic potential.

Author details

Melih Dagdeviren

Address all correspondence to: melih.dagdeviren@ege.edu.tr

Department of Biology, Faculty of Science, Ege University, Izmir, Turkey

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