Transgenic Mouse Overexpressing Spermine Oxidase in Cerebrocortical Neurons

Subjects: Neurosciences

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Polyamines (PAs) are organic polycations ubiquitously present in living cells. The main PAs in mammalian cells include putrescine (Put), spermidine (Spd), and spermine (Spm), and their acetylated forms, N1-acetylspermidine and N1-acetylspermine. Polyamines are involved in many cellular processes, and their content in mammalian cells is tightly controlled. Among their function, these molecules modulate the activity of several ion channels. Spermine oxidase (SMOX) specifically oxidizes spermine, a neuromodulator of several types of ion channel and ionotropic glutamate receptors, and its deregulated activity has been linked to several brain pathologies, including epilepsy. The Dach-SMOX mouse line was generated using a Cre/loxP-based recombination approach to study the complex and critical functions carried out by spermine oxidase and spermine in the mammalian brain.

Keywords: Polyamines ; SMOX ; glutamate excitotoxicity ; reactive astrocytosis

1. Polyamines Metabolism

Polyamines (PAs) are organic polycations ubiquitously present in living cells. The main PAs in mammalian cells include putrescine (Put), spermidine (Spd), and spermine (Spm), and their acetylated forms, N^1-acetylspermidine and N^1-acetylspermine (**Figure 1**).

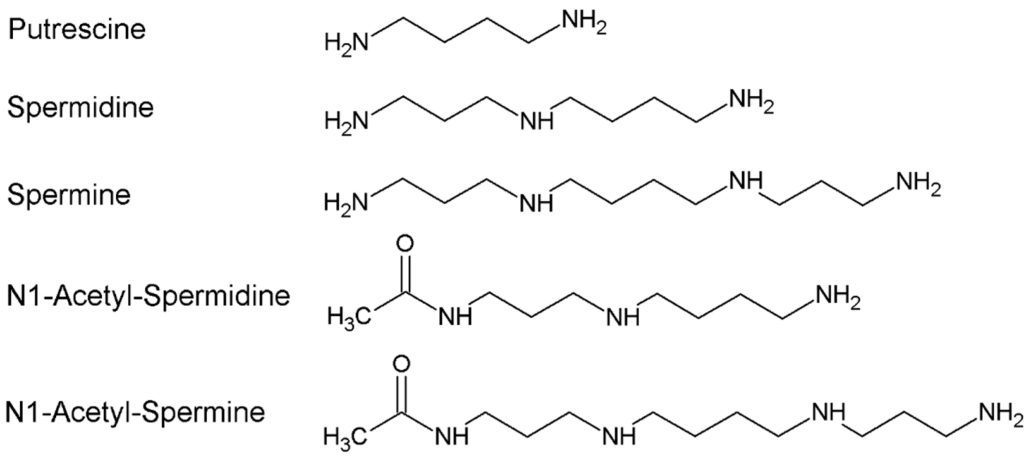

Figure 1. Polyamines and their acetyl derivatives.

The PA content in mammalian cells is tightly regulated [1][2][3] and it has been linked to important cellular roles. Polyamines are involved in the synthesis of proteins and nucleic acid and in the maintenance of their structure, in the regulation of the activity of ion channels, in cell proliferation, differentiation, and apoptosis, as well as in protection from oxidative damage [1][2][3]. Altered PA cellular levels have been reported in several pathological conditions of the Central Nervous System (CNS). One of the best examples is the low level of Spm observed in the Snyder–Robinson syndrome, an intellectual disability disorder with movement disorder and seizures, due to a rare mutation of the Spm synthase gene in the X-chromosome [4]. Alteration in the PAs' synthesis and metabolism have been reported to be correlated with suicidal behavior [5]. Multiple symptoms including neurological abnormalities are also reported in rodent models of altered PA synthesis and catabolism [6][7]. Notably, PAs have been suggested to counteract cognitive impairment in animal models by activating autophagy and mitochondrial function, and high dietary Spd intake correlated with lower risk for cognitive impairment in humans. Polyamine biosynthesis is carried out by the action of four enzymes: S-adenosylmethionine decarboxylase enzyme (AdoMetDC), ornithine decarboxylase enzyme (ODC), spermine synthase (SMS), and spermidine synthase (SPDS) [8]. While a PA catabolic pathway is dependent on the activity of three enzymes: N^1-acetylpolyamine

oxidase (PAOX), spermidine/spermine N^1-acetyltransferase (SAT1), and spermine oxidase (SMOX) [7][9][10][11]. **Figure 2** depicts a schematic representation of the PA metabolism.

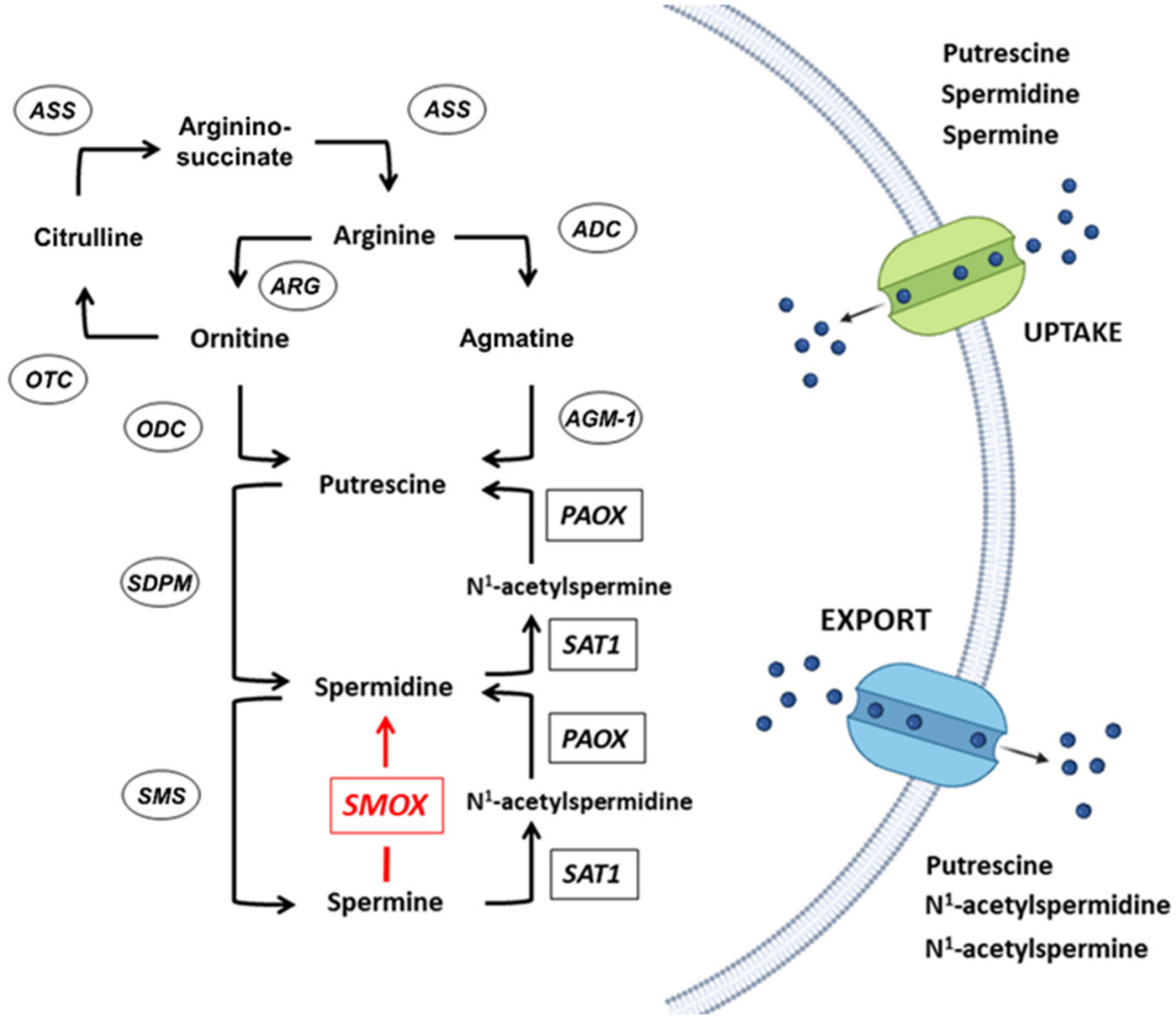

Figure 2. Polyamine's metabolism. Enzymes involved in PA biosynthesis and catabolism are encircled and boxed, respectively. ADC, arginine decarboxylase; AGM-1, agmatinase; ARG, arginase; ASS, arginino-succinate synthase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; PAOX, N^1-acetylpolyamine oxidase; SAT1, spermidine/spermine N^1-acetyltransferase; SMS, spermine synthase; SPDS, spermidine synthase. Spermine oxidase (SMOX) is highlighted in red and is overexpressed in the DACH-SMOX transgenic mice model, (ARG), (AGM1), (ADC), (OTC).

2. The SMOX Overexpressing Mouse: An Animal Model of Chronic Spm Catabolism Activation

The enzyme SMOX specifically recognizes Spm as a substrate to produce Spd, with the production of hydrogen peroxide (H_2O_2) and 3-aminopropanal (3-AP) (**Figure 3**) [12][13][14].

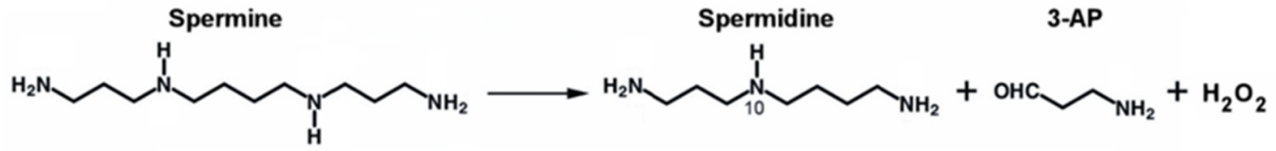

Figure 3. Spermine oxidase enzymatic reaction. The substrate spermine is oxidized to produce spermidine, 3-aminopropanal (3-AP), and hydrogen peroxide (H_2O_2).

The SMOX enzyme is expressed in various tissues, mainly in brain and skeletal muscle, and regulates the Spm/Spd ratio to keep the cellular PA content balanced [7][15]. Apart from its role in the basal PA metabolism, the SMOX substrate Spm has an important function in the brain, since intracellular Spm is also a neuromodulator responsible for intrinsic gating and rectification of strong inward rectifier K^+ channels (Kir) by directly plugging the ion channel pore [16][17][18]. Moreover, the intracellular level of Spm, by plugging the receptor channel pore, can also cause inward rectification of some subtypes of

alpha-amino-3-hydroxy-5-methyl-4-isoxazole- propionic acid (AMPA) and kainate Ca^{2+}-permeable receptors in the CNS [19][20]. Furthermore, extracellular Spm has multiple effects at the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors, increasing the intensity of NMDA receptor currents and voltage-dependent blocks [16][17][18]. The oxidation product of SMOX Spd is also a neuromodulator, but less potent than Spm since it binds the same channel receptors with much less affinity [17]. Several works have highlighted new roles for Spd that protect from age-induced memory impairment acting directly at synapses by means of an autophagy-dependent homeostatic regulation [21] or enhancing eEF5/EIF5A hypusination, cerebral mitochondrial function, and cognition in aging Drosophila melanogaster and mice [22]. It has been demonstrated that Spd induces autophagy in several model systems, including rodent tissues and cultured human cells [23][24][25]. The integrity of the autophagic system has been suggested to be crucial for the Spd-mediated protection from age-associated presynaptic active zone changes and increase in the active zone scaffold components in D. melanogaster [26]. Furthermore, SMOX activity not only controls Spm/Spd cellular ratio, but it is also a source of cellular redox alteration by producing H_2O_2, a reactive oxygen species (ROS). Hydrogen peroxide can also modulate brain Long-Term Potentiation in a dose-dependent manner, but an excessive increase of it can result in learning and memory impairment [27]. Spermine oxidation by SMOX is also responsible for secondary tissue damage, due to the generation of 3-AP, which spontaneously converts into acrolein [11][28]. Noteworthy, the production of acrolein in the injured brain is a further source of inflammation and apoptotic cell death; in line with this, a decrease in Spm content and an increase in plasma protein conjugated-acrolein (PC-Acro) could be considered a good marker for brain infarction [28]. Furthermore, in stroke patients, the high levels of SMOX, PAOX, and PC-Acro in plasma correlates with the stroke size [29][30][31][32][33][34]. A mouse model conditionally overexpressing SMOX in the neocortical neurons with a CD1 background, formerly JoSMOrec, now DACH-SMOX, has been engineered (**Figure 4**) [12][35].

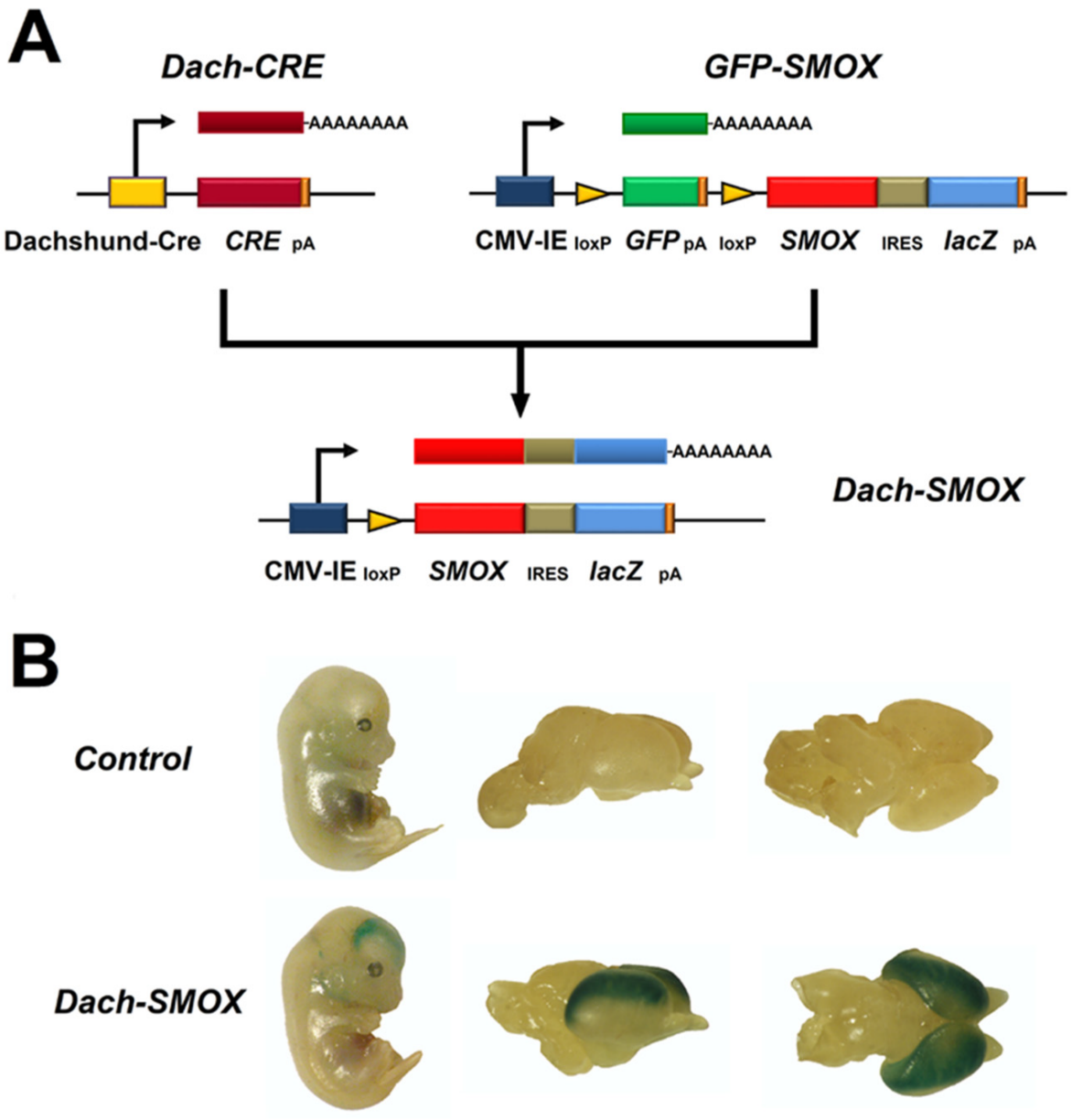

Figure 4. Scheme of Dach-SMOX mouse generation. (**A**) CRE recombination scheme between Dach-CRE and GFP-SMOX to obtain Dach-SMOX mouse lines. (**B**) Left-side, LacZ staining of wholemount at E12.5 mouse developmental

stage of Dach-SMOX and control embryos; right-side, LacZ staining of brains at E14.5 mouse developmental stage of Dach-SMOX and control embryos.

The Dach-SMOX mouse line is a chronic model of excitotoxic/oxidative injury and neuron vulnerability to oxidative stress and excitotoxic insults [36][37][38]. Notably, the mice revealed to be a chronic model of increased susceptibility to seizures that might help to understand neuron vulnerability to insult as well as epileptogenesis. This mouse genetic line provides a potential model to be further exploited, in addition to simple acute epilepsy animal models, for new pharmacological approaches to cure epilepsy. The results obtained from the study on SMOX overexpressing mice are of relevance also in the scenario of the increasingly recognized importance of astrocytes in brain function [39][40][41], which is now shifting from a neurocentric to a neuro-astrocentric view [42]. In fact, chronic overexpression of SMOX in cortical neurons of Dach-SMOX mice severely affects their astrocyte morphology and function, and heavily influences cerebrocortical synapse functioning [43]. The findings support roles for endogenous PAs in maintaining neuron–astrocyte cross-talk and in neuroprotection [44], and that an imbalance of PA synthesis and flux can alter the neuron–glial communication in the brain [16][44][45]. Consistently, accumulating evidence indicates that PAs are synthesized in neurons, released from neurons into the extracellular space, and preferentially accumulated in glial cells [46] from glial cells, they can then be secreted back to neurons [45]. Indeed, in SMOX-overexpressing mouse changes of PA metabolism and H_2O_2 overproduction co-occurring in cerebrocortical neurons seemingly to affects astrocytes, and in turn, neurons. In particular, reactive astrocytosis and neuron loss are the main effects of the chronic activation of Spm catabolism in cerebrocortical neurons in Dach-SMOX mice, together with chronic oxidative stress and excitotoxicity [36][37][38]. The main changes and their molecular mechanisms in the Dach-SMOX line are highlighted in the following paragraphs.

3. Reactive Astrocytosis

The relevance of neuron-astrocyte networks to the control of signal transmission and the regulation of brain function is widely recognized [39][40][47][48]. Astrocytes provide neurons with energy substrates, nutrients and neurotransmitter precursors, structural support around synapses, and buffering of the excess of K^+ and neurotransmitters in the extracellular space [49][50][51][52]. Astrocytes regulate extracellular glutamate concentration by balancing its uptake through the glutamate transporters EAAT1 and EAAT2, and its release and uptake through the antiporter cystine-glutamate exchanger xc^- [53][54]. Furthermore, astrocytes and, in particular, the astrocytes processes, can release glutamate in a Ca^{2+}-dependent vesicular or Ca^{2+}-independent ways [36][37][55][56][57][58][59][60][61][62][63][64]. The perisynaptic astrocytic processes (PAPs), which envelop synapses and are primarily involved in astrocyte-neuron communication at tripartite synapses [56][61], display rapid movements and Ca^{2+} elevations in response to neuronal activity [65], and regulate coverage of synapses, synapse plasticity, and the interstitial space volume [66]. In response to brain injury, astrocytes undergo morphological, molecular, and functional remodeling, also known as the so-called reactive astrocytosis [50][67]. The contribution of reactive astrocytes to CNS diseases and repair is matter of debate, and both detrimental and neuroprotective actions have been attributed to reactive astrocytes [68]. Reactive astrogliosis in the cerebral cortex of Dach-SMOX mice is indicated by an increased number of astrocytes and morphological cellular changes consisting in hypertrophy and wide ramification [12][36]. Consistent with the presence of reactive astrocytes and a neuroinflammation condition [69], a relative abundance of astrocyte processes versus nerve terminals was observed in Dach-SMOX mice, with the increase in GFAP-positive particles, and a reduction of synaptophysin-positive particles [36], together with increased levels of the astroglial markers ezrin and vimentin [43]. Indeed, vimentin is a potential marker for reactive astrocytes [67][68] of relevance in the control of the function of astrocytes and astrocyte processes in reactive astrocytosis [70]. Furthermore, an increase in ezrin, a protein preferentially localized in the fine PAPs unsheathing synapses [71], specifically indicates changes of these fine processes. This might have consequences on the synapse function, as PAPs are involved in glia-synaptic interactions, and ezrin is required for PAPs motility and regulation of synapse coverage [72]. Indeed, ezrin participation in both neuroprotective and neurotoxic activities of the reactive astrocyte processes is a matter of intense study [71]. Altogether, these findings point out a typical remodeling of reactive astrocytes in response to chronic activation of PA catabolism. Astrocytes in the cerebral cortex of Dach-SMOX mice may become reactive, possibly responding to overproduction of H_2O_2 due to SMOX overexpression, and/or as a consequence of neuron impairment. In fact, oxidative stress and inflammation are significant factors promoting reactive astrocytosis [73], and at the same time, activated astrocytes are capable of generating ROS [74]. In addition, synaptic neuronal activity is crucial to maintain healthy astrocytes by trophic signaling that can regulate astrocyte function [65]. It can be hypothesized that PAs are among these trophic factors produced in neurons, then released and accumulated in glial cells [16][44][45]. In turn, reactive astrocytes may have detrimental effects on neurons, contributing to the dysregulation of synapse functioning [43]. The processes of reactive astrocytes in Dach-SMOX mice undergo the following modifications that might impact on glutamatergic synapse function: reduced Spm content, expression of AMPA GluA2-lacking receptors linked to Ca^{2+} entry

and activation of glutamate release, increased xc^- transporter function and increased glutamate in-out transport, reduced expression of EAAT1 and EAAT2.

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