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Review article

Role of perivascular and paravascular drainage of Aß, iron ions, and waste products from the brain

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Abstract

***Objective*:** The analysis of the intracerebral accumulation of amyloid beta (Aβ) and iron ions, as well as its crucial role in the pathophysiology of Alzheimer's disease (AD), represents the focal point of recent investigations related to genetic, molecular, pathophysiologic and clinical aspects of this wasting chronic and lethal neurodegenerative disease. AD is dominantly connected with the brain, the accumulation of Aβ, iron ions, and a whole complex of waste products, related primarily to intensive cerebral oxidative processes. Therefore, adequate drainage of these substances out from the brain is essential, but as the brain does not have a standard lymphatic system like other body organs, the drainage problem becomes very complex. Intensive investigations in this field, aimed at discovering other drainage pathways, have shown a complex of other nonconventional pathways, including the perineural route, two atypical lymphatic routes, drainage by BBB and BCSFB, and especially the so-called perivascular and paravascular pathways, both closely connected to the cerebral vasculature. The development of the investigation technology related to radioisotopic tracers injected into the brain, the electronic imagery of brain structures, and the haematic and fluid flow, have all greatly contributed to the understanding of the drainage problem, and possible adequate therapy of drainage disturbances. This study does not analyze all aspects of the drainage events; its aim is a comprehensive analysis of the brain vascular system drainage, *i.e.* the para and perivascular drainage and its pathophysiology.

***Methods*:** Using a comprehensive spectrum of relevant literature related to the process of the drainage of Aβ, iron ions, and a series of waste products generated by metabolic and oxidative events, as well as by other biochemical reactions, this study presents a review of crucial processes whose damages have a strong effect on the onset and course of AD. Besides the available materials, I have also considered additional consultations with prominent investigators in this field.

***Results*:** Obtained results show the great importance of the appropriate drainage of the mentioned compounds. Every elevation of their intracerebral concentration, especially of Aβ and iron ions, immediately reflects on the course of AD and its deterioration.

***Discussion*:** This section presents the investigation results of a number of prominent experts interested in the mentioned problems. Although there is great progress in defining the exact causes and routes of the drainage, there are still a number of unsolved moments which have to be cleared.

***Conclusion*:** Alzheimer's disease is a severe, chronic and lethal neurodegenerative disease, with as yet an inadequately explained etiology. However, further investigations are necessary to explain the oxidative stress induced by the extracellular accumulation of Aβ and metal ions (especially iron ions), as well as the not yet sufficiently explored role of their drainage routes.

***KEY WORDS*:** Alzheimer's disease, amyloid beta (Aβ), iron ions, drainage from the brain

Introduction:

Before the description and comprehensive analysis of the two main drainage pathways for the clearance of amyloid beta (Aβ) peptides, iron, and waste from the brain of patients suffering from Alzheimer's disease (AD), it is necessary to mention some important facts about this disease.

AD, otherwise linked with the aging process and age, following the increasing absolute and relative growth of the worldwide elderly population, has shown an evident rise in its incidence and prevalence. This serious neurodegenerative disease, labeled with a chronic and progressive course and with an unavoidable lethal outcome at the end, is during its

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course marked by an increasing loss of memory, thinking and speaking. At the end the patients become completely weak and completely dependent on someone else's care and help [1].

A number of earlier and recent investigations show that this serious disease, actually with a polygenetic etiology, is crucially connected to the pathologic extracellular accumulation of Aβ which are increasingly produced by neurons, and the growing amount of Fe^{3+} and Fe^{2+} iron ions. Aβ, a composed polypeptide, is generated by the proteolytic degradation of amyloid precursor protein (APP) peptide belonging to the neuronal membrane. Separating from this membrane, Aβ enters the extracellular space (neuropil) and binds Fe^{3+} ions (ferric ions) on its β1 arm, located on the strongly defined region MBD (metal-binding domain, His13-His14). Generally, Aβ has a strong affinity to Fe^{3+} ions. AD exists in two essential forms: early-onset AD (EOAD; 5% of all cases, occurs before 65 years of life), and the late form, late-onset AD (LOAD; 95% of all cases, occurs after 65 years of life). The clinical form of both units is almost the same [2-4].

During the Aβ monomer aggregation process, which is otherwise the crucial feature of this deteriorative disease, in the beginning, the monomers' mutual approach is oblique, unparallel and incongruent. The resulting interaction between the β2 strand of the Aβ incoming monomer (Aβ1) and β1 strand of the fixed monomer (Aβ2), on the top of the already formed protofilament, can occur when the distance between the two reactive points MetS35 (Aβ1, β2 strand) and MBD (Aβ2, β1 strand) becomes less than 18Å. In this case the phenomenon of electron "hop" from the S of MetS35 to the Fe^{3+} on MBD (reduction). Following this reductive reaction occurs the Fenton reaction (Fe^{2+} becomes oxidized by hydroxide peroxide H_2O_2, with the generation of Fe^{3+} (ferric ion), ^{-}OH (hydroxyl ion), and *OH (very toxic and aggressive hydroxyl radical) occurs. The reaction formula is: $Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{-}OH + {}^{*}OH$. The generated *OH, otherwise a strong oxidant and an electron stealer, immediately attacks the neighboring molecular structures causing their destruction. All this shows that in AD pathophysiology, in the center of the destructive phenomena, lies the oxidative process, induced by a series of transitional metal ions, among them primarily iron ions [1, 2, 5-9].

Laboratory tests and other sophisticated analyses show that during AD development an increasing accumulation of waste products occurs in the cerebral interstitium, among them especially Aβ and iron ions. Unfortunately, the brain does not have an adequate lymphatic drainage mechanism, like other organs in the body, necessary for the clearance of these products. However, this function, optimal to maintain the balance between the accumulation of these harmful products and their degradation and drainage out from the brain, is realized through special drainage pathways, among them the peri and paravascular routes which are the topic of this review. The disbalance or failure of the mentioned drainage, which is unfortunately typical for AD, leads to the progression of the disease, deterioration of symptoms and lethal end [10].

For a better understanding of the drainage processes of Aβ and iron ions, it is necessary to emphasize that the elevation of their concentration in the neuropil has a strong genetic background. The analysis of their role surpasses the borders of this study [1, 2, 5, 6].

Without entering into the explanation of iron uptake from food, its travelling by blood, and passing across the blood brain barrier (BBB), which is presented in detail in my earlier papers (see Ref. 6 and Ref 7), my aim in this study is only to analyse the recent knowledge about iron clearance from the brain and its drainage through two main pathways, the para and perivascular pathways.

At the beginning of the study, with a small deviation from the drainage problems, it is very important to emphasize that Aβ has a strong toxic effect on the oligodendrocytes (OLGs), which otherwise strongly synthesize and release transferrin (Tf) and myelin. OLGs have an intensive metabolism, low concentration of protective glutathione (GSH), and are full of ferritin and iron. By the effect of Aβ, OLGs are destroyed subsequent to mitochondrial dysfunction. The decline of Tf and myelin production also occurs with a consequent decline in the Tf-Fe^{3+} complex and its influx into the cells. The concentration of Fe^{3+} in the neuropil rises. The decline in myelin production has a very bad effect on neuronal function. OLG destruction is a strong stimulus for macrophage activation and their migration. In any case, they phagocytize the damaged cell structures and secrete the ferritin. The result is the destruction of OLGs and the exit of a great number of iron ions and ferritin from these OLGs. Although Tf is also synthesized in the choroid plexus, this can probably hardly compensate for its great loss induced by the OLGs' destruction. The confirmation of this fact lies in the marked low Tf concentration in the brain of AD patients. In AD the values of Tf are also diminished in blood serum [9-13].

Aβ has a strong, experimentally confirmed, affinity for Fe^{3+}. After the interaction between MetS35, Aβ1, β2 strand and MBD, Aβ2, β1 strand (Fe^{3+} reduction), the generated Fe^{2+} immediately enters the mentioned Fenton reaction, with the consequent generation of great quantities of *OH. The result is serious oxidative damage to the neighboring molecular structures [2, 5, 8].

It is clear that the manifestly protective mechanisms, which degrade and drain this great amount of generated and aggregated Aβ, are important for the course of AD. On the other hand, it is evident that the strong accumulation of iron ions needs adequate drainage as well. All the mentioned facts can explain the growing interest of investigators in the structure and function of brain drainage pathways.

Discussion

The analysis of Aβ drainage and a number of metabolic waste products from brain cells as well as of other biochemical processes requires a detailed description of paravascular and perivascular pathways which are included in these events. It is necessary to emphasize that this study is not involved in the description of other known pathways, which will also be mentioned. Paravascular and perivascular pathways are being increasingly explored. The comprehension of their structure and function gives new hope for better and more effective AD therapy. This study will first give a survey of the paravascular pathways, and the perivascular pathways will be elaborated later (***Fig. 1, 2***).

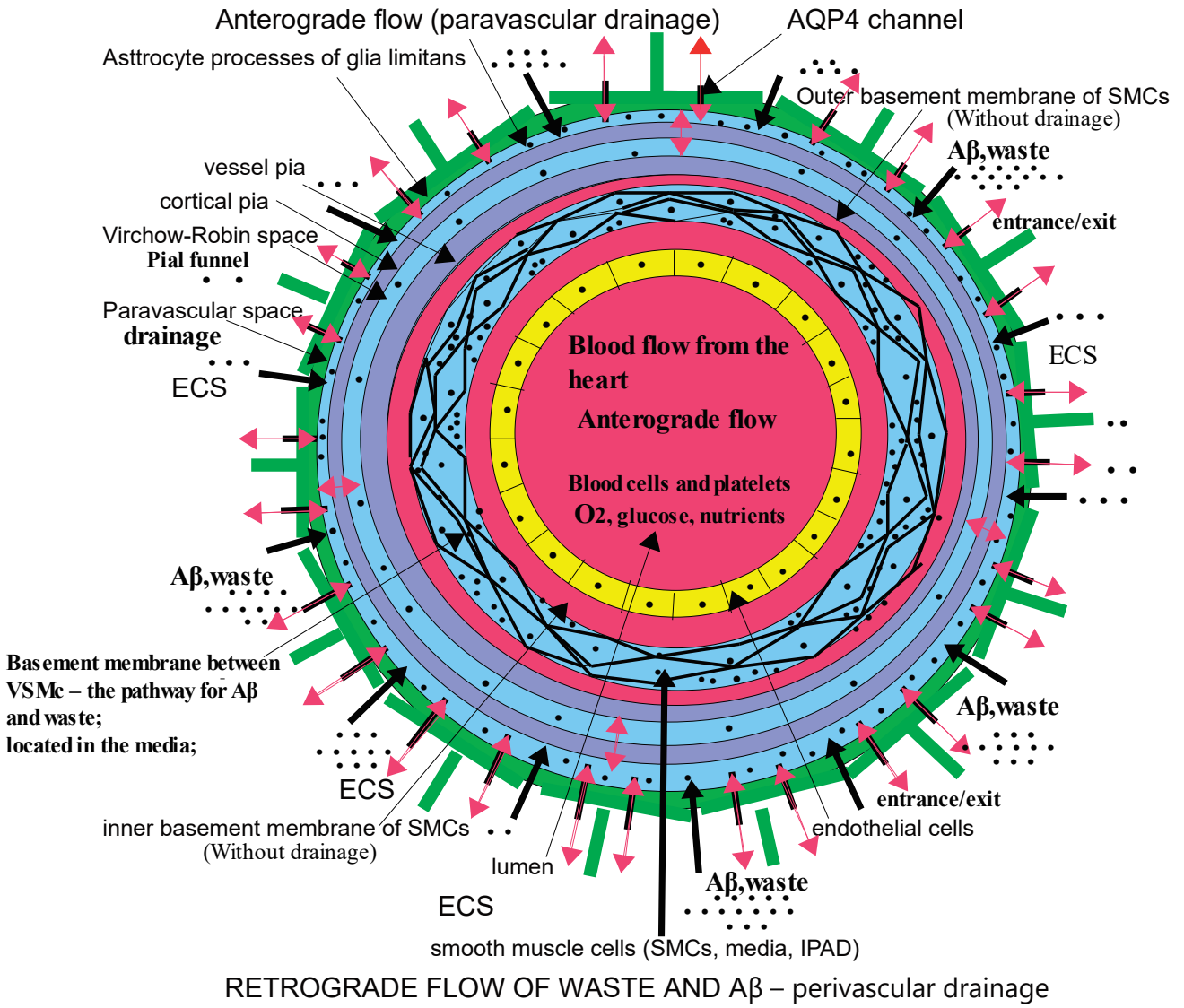

Fig. 1. **Schematic presentation of the penetrate artery cross section.**

The cross section of the cerebral parenchymal penetrating artery. By different colors the essential artery layers are visible, including the IPAD pathway and paravascular drainage pathway. Dark green color designates the astrocytic endfeet, and between them the black arrows show intercellular astrocytic endfeet clefts. Small black rectangles, covered with bidirectional arrows, present the AQP4 channels, important for the entrance into the artery wall and the exit from it, of a lot of solutions and Aβ peptides. The big black arrow shows the most important layer in the artery wall, the media, and in its structures, located between vascular smooth muscle cells, the perivascular drainage pathway (black points in the layers between the mentioned cells). Black points in the ECS represent Aβ and waste. Between the cortical and vessel pia, there is the VRS. On the outer side of the cortical pia, in light blue color, the paravascular drainage pathway is visible. Cross section presents the situation on the middle level of the VRS. SMCs are in the relaxed phase of the vasomotion wave; MCA at its origin has an outer diameter ranging from 2.5-4.0 mm with a mean of 3.35 mm. AQP4, aquaporin-4; IPAD, intramural periarterial drainage; Aβ, amyloid-β; ECS, extracellular space; VRS; Virchow-Robin space, VSMCs, vascular smooth muscle cells; MCA, middle cerebral artery.

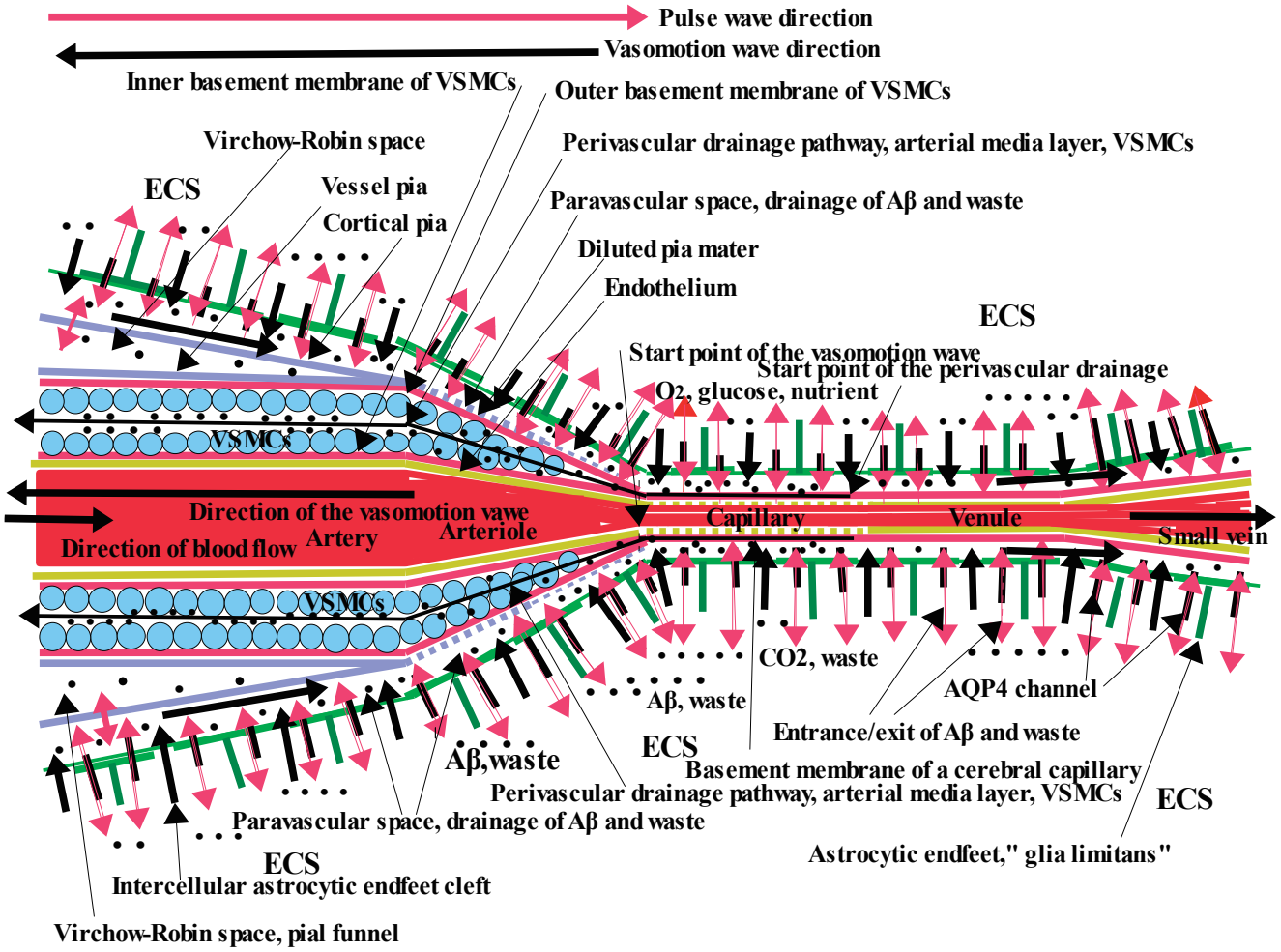

Fig. 2. **Schematic presentation of the paravascular and perivascular drainage pathways (longitudinal section).**

The perivascular drainage pathway (PDP) located along the cerebral artery between the outer and inner basement membranes of VSMCs. At the arteriolar level, it is located also between these two layers. At the capillary level, this pathway is located in the capillary basement membrane. Aβ and a lot of waste products enter into the capillary basement membrane from the ECS. At this level, there are not two individual basement membranes because they have joined together into one, and the same situation occurs at the venule and vein levels. In the artery, the arrows which indicate the direction of blood and vasomotion wave are visible. These two directions are opposite. At the end of arterioles and at the beginning of capillaries, the location of the precapillary sphincter is visible. This location is in fact the starting point of the vasomotion wave. The paravascular drainage pathway is located in the space between the cortical and arteriolar pia on the inner side and "glia limitans" on the outer side. The distal part of PDP along the arteriole, on the inner side, is confined by the diluted and not clearly visible pia, and on the outer side by the earlier mentioned "glia limitans". Around the capillaries, venule, and vein, this space is confined by "glia limitans" and the basement membrane of venules and veins. The direction of Aβ and waste particles in this pathway have the same direction as the blood flow (forward direction). AQP4 channels located on the astrocytic endfeet, have a bidirectional flow (entrance/exit). The driving force for perivascular drainage is linked with the vasomotion wave and is not dependent on heartbeats and pulse waves. VSMCs, vascular smooth muscle cells; Aβ, amyloid-β; ECS, extracellular space; AQP4, aquaporin-4; Aβ, amyloid-β; ECS, extracellular space; VSMCs, vascular smooth muscle cells.

Paravascular drainage

Bedussi B *et al.*[14] in their study immediately emphasize that the clearance of waste products from the brain is of crucial importance. They especially emphasize the paravascular channels around the blood vessels and arterial pulsations as the driving forces for this flow. They point out that this flow has a pulsatile character and that it is induced by the cardiac cycle, that has a dominantly anterograde direction, and its resistance is markedly low due to its relatively greater width. The authors of the study have tested the flow by fluorescent microspheres (1 μm fluoSpheres, Ex. 495/Em. 515). The moving of these particles has a close correlation with the oscillating blood velocity and with changes in arterial diameter. These pulsations, according to the authors, induce the driving forces of the investigated transport. The net effect of the particles' direction was anterograde. During the complete systole the particles were moving regularly parallel with the direction of the flow, and their direction was the same also during the initial part of the diastole. During the second part of the diastole, the microspheres had an inverted direction. The mean velocity of the flow was 17 ± 2 μm/s. The paravascular space (PVS) around leptomeningeal arteries had direct relation (the vessel pia constitutes its boundary with the artery wall) with the subarachnoid space (SAS), and at the level of the brain parenchyma (penetrant arteries: 20-50 mm long and 100-200 μm wide), the PVS was separated from SAS by the markedly permeable pia and on the initial part of the mentioned level by the pial funnel (Virchow-Robin space). PVS was obviously wider around the arteries in comparison to the veins. Paravenous space was separated from SAS by very permeable pia. Particle transport was significantly weaker around the veins as compared to arteries. The undertaken investigation was carried out in mice (Male C57BL/6JO1aHsd mice) preliminarily anesthetized with an intraperitoneal injection using exactly defined anesthetics. By the infusion of microspheres into the cisterna magna, and subsequent imaging of the middle cerebral artery (MCA) and its branches, using a special technique, a clear image of pulsatile anterograde movement of these particles was obtained along the outer wall into the layer about 20 μm wide. Their experiments, by imaging of the MCA, have shown that after a delay of approximately 45 min (after the microspheres are thrown into the cysterna magna), microspheres began appearing around the MCA branches. The greatest portion of microspheres was moving anterogradely in relation to the direction of the blood flow, close to the arterial wall. Further, from the wall their number progressively declined, but their movement was also anterograde. The width of the exactly determined main periarterial microspheres moving pathway was approximately 20 μm. The authors determined this value as the width of the paravascular drainage pathway. A special highly sophisticated technology was used to obtain adequate imagery of the MCA and its branches, as well as the microspheres' movement. However, the description of this technique exceeds the range of this study. On the inner side of this pathway around the meningeal arteries, there is a pial sheath and on the supposed outer side there are no astrocytic endfeet, but only CSF. The astrocytic endfeet occur at the level of penetrating arteries and brain parenchyma (***Table 1***).

The close contact between the moving microspheres and leptomeningeal walls, as well as the only 20 μm wide line of the preferred paravascular pathway, can probably be explained by the influence of electrostatic interactions. Microspheres have a positive electrostatic charge, and collagen molecules, the important components of the arterial wall structure, are electronegative. However, after personal communication with Mr. Erik Bakker, the member of this research group, the influence of the electrostatic forces was eliminated. Here is his explanation: The microspheres were preincubated before the injection with albumin, which is negatively charged. So, the positive charge from the microspheres would have probably been previously neutralized by the albumin. In summary, the authors have concluded that with great probability, it is possible that the arterial pulsations were generated from the heart action, and their interaction with the rigid skull, as well as the respiratory movements, constitute the cause of the driving force for the paravascular flow[14].

The investigated pathway is limited on its inner side (towards the arterial wall) by the vessel pia in the region of SAS, and after the perforation into the brain parenchyma, it enters the Virchow-Robin space, *i.e.* the funnel of the Virchow-Robin space (VRS). After the passage through the cortical pia (the outer part of the funnel) the pathway enters the already existing space, limited on the inner side with the cortical pia and on the outer side with the glia limitans[15]. The glial membrane is composed of a great number of mutually attached astrocytic endfeet. Distally, the pia is subjected to the connecting of the vessel and the cortical part, by indefinite alterations, and gradually disappears in the direction towards the arterioles. The investigated space at the capillary level on the outer side is limited with the glial membrane and on the inner side with only the capillary basement membrane and capillary endothelium. Interstitial fluid (ISF) and Aβ enter this pathway at the capillary level through the aquaporin-4 (AQP4) channels on the endfeet[16, 17]. The movement of these elements is anterograde. The proximal border of the level of this pathway is formed by the pia and basement membrane of glia limitans which form the border of SAS towards the brain parenchyma. Glia limitans has a number of relatively sufficiently wide clefts (intercellular astrocytic endfeet clefts) through which ISF and Aβ enter into the PVS. On the capillary level, the mentioned compounds enter the capillary basement membrane, and then along this membrane, they continue to move proximally as the perivascular pathway. When they arrive at the arteriolar level, they enter into the space between the vascular smooth muscle cells (VSMCs) and continue further on moving retrogradely. It is important to emphasize that VRS also enables the clearance of waste particles from the brain, as well as the interaction of the systemic immune system with important cerebral functions (***Fig. 2***).

Bacyinsky A *et al.*[18] in their study present a comprehensive analysis of the PVS, in other words, the "glymphatic" pathway, as the system of great importance for the clearance of waste material from the brain. According to them, the cerebrospinal fluid (CSF), after passing through the permeable pia, enters into the PVS and mixes with the present ISF. ISF has previously entered into PVS through AQP4 channels and intercellular astrocytic endfeet relatively wide clefts. Moving distally by this route, waste products, as well as Aβ, pass along the capillary basement membrane and enter into the PVS of the venous drainage (paravascular space of draining veins). By this route, the waste passes on the outer side at

Table 1. Driving forces for paravascular drainage.

Different parameters	Description of events
Starting point of the pulse wave	Systolic heartbeat (left ventricle contraction)
Direction of the pulse wave	Anterograde direction (opposite to the heart) along with the arterial system up to the arteriolar, capillary and precapillary sphincters
Cause of the pulse wave	Systolic myocardial contraction induced by bioelectrical forces
Reflecting wave: the retrograde direction; in the brain circulation, this wave is much weaker than the wave in the peripheral circulation, and the accompanying retrograde flow of Aβ and waste is minimal	Encounter between the incoming pulse wave and the resistant distal physiological barrier composed of arteriolar, capillary, and precapillary sphincters; the resistance induced by this barrier is not as strong as the barrier induced in the periphery
The driving force for this type of drainage is induced by the hydrostatic gradient between the strong arterial pulsations and lower pressure in the paravascular space and strong expression of AQP4 channels;	The relatively strong movement of Aβ, iron ions and waste along this pathway
Direction of Aβ, iron ions, and waste particles along this pathway	Anterograde
Associated auxiliary factors	respiratory excursions, vasomotor wave fluctuation, the body position

These facts require future investigations.

the level of neuropil, limited by the basement membrane of glia limitans, and on the inner side limited by the basement membrane of the vein, and by the thin media with some VSMCs and with endothelium. After the entrance into the SAS, this route loses the outer layer of glia limitans and receives the circulatory layer of pia. After entering the dura level, the vein endothelium and the basement membrane continue in the same layers of the sinus sagittal superior. The pia disappears [16-18].

Further analysis of PVS by the same authors shows that the CSF, previously having entered into this space from the SAS through the obviously permeable pia, exits from PVS through the AQP4 channels and astrocytic intercellular endfeet clefts (diameter 20-50 μm), and enters the extracellular space of the brain parenchyma (ECS). While passing through the ECS, CSF mixes with ISF, enters again into the PVS through the AQP4 channels and astrocytic endfeet of the basement membrane of glia limitans near the drainage vein wall. The fluid mixture, containing Aβ, lactate, and other solutes, continues moving to the sinus sagittal superior and venous bloodstream [18]. It is necessary to emphasize that some studies have not found waste particles in the PVSs.

Analyzing the movement of small fluorescent tracers, Iliff JJ *et al.* [17] have determined that CSF enters the brain parenchyma along the paravascular pathway and here mixes with ISF and other dissolved material. CSF then returns back into the PVS and is eliminated from the brain (superior sagittal sinus). The authors present fine imagery of CSF movement from its generation in the choroid plexus of ventricles up to the entrance into the SAS through foramina of Luschke and foramen Magendi. From SAS, CSF can be reabsorbed into the blood flow through arachnoid granulations, or it travels to the cervical lymph nodes through

perineural pathways of cerebral nerves. The greatest part of CSF is reabsorbed passing through the permeable pia and paravascular pathway into the cerebral parenchyma, where, as it has been emphasized earlier, it mixes with ISF (CSF-ISF exchange) and through AQP4 water channels this mixture again enters the lymphatic system and through this system comes into the superior sagittal sinus and the blood [16, 17].

Paravascular venous space is limited by the venous wall on the inner side, and by the glia limitans on the outer side. In the venous vasculature, there is no perivascular space. The fluid flow in the venous PVS is anterograde (the direction towards the entrance into the sagittal superior sinus). By entering deeper into the cerebral parenchyma and by their narrowing with the formation of capillaries, arterioles lose the smooth muscle layer otherwise embedded between the outer and inner basement membranes of SMCs.

These two membranes are distally practically integrated into one membrane which stretches distally and encircles the capillary endothelium. The very permeable and poorly resistant capillary membrane permits the entrance of ISF and Aβ into its interior and creates the conditions for the beginning of the perivascular drainage. The pia, which after the connecting of the cortical and vessel pia, and after the closing of the VRS, is much thinner and inconsistent, practically disappears in the direction towards the capillaries. Between the glia limitans and the capillary basement membrane, there is now only a narrow PVS [15]. The essential question is what is the driving force of the paravascular flow. Engelhardt B *et al.* [23] consider that the higher pressure arterial pulsations as well as the strong expression of AQP4 channels are crutial for the low pressure in the paravenous space. In this way, the hydrostatic gradient is generated and it starts the anterograde flow and ISF drainage. It seems that this mechanism is supported by respiration excursions, vasomotion wave fluctuations, and body posture. However, recent investigations raise doubts about the mentioned views (***Table 1***) [16, 17].

Diem AK *et al.* [24, 25] comprehensively explain these doubts, demonstrating that arterial pulsations are not strong enough to produce adequate drainage velocities. They also give a comprehensive description of the VRS and claim that this space has no role in Aβ drainage from the extracellular space (ECS) [15].

Perivascular drainage

At the beginning of their study, Morris AWJ *et al.* [19] assert that due to the lack of conventional lymphatic drainage of ISF and dissolved materials in it, the fluid can find the draining pathway up to the cervical lymphatic nodes, along the basement membranes inside the walls of cerebral capillaries and arterial tunica media (cerebral vascular basement membrane pathway). By intracerebral injection containing dissolved biotinylated or fluorescent Aβ, as well as gold nanoparticles, into the mouse hippocampus, and by the analysis using transmission electron microscopy, the authors confirm the spread of the mentioned Aβ particles within the adequate extracellular spaces, capillary basement membranes and arterial tunica media. The presence of gold nanoparticles has not been found in the capillary basement membranes, which indicates that they have not entered the periarterial space. They have exclusively been found in the paravascular space. However, the investigated perivascular pathway is too narrow for the passage of the antigen carrying cells as well as for lymphocytes. The disorder of this pathway often leads to cerebral amyloid angiopathy (CAA). The study undoubtedly supports the hypothesis that the basement membranes are the main pathway for the transport of fluid and a number of dissolved particles in it, especially soluble Aβ, out of and into the brain parenchyma. Parallel with the course of the aging process, this pathway becomes significantly less capable of draining Aβ out from the brain. This creates the optimal condition for Aβ extracellular aggregation, plaque formation, and CAA development. The authors also emphasize the great importance of AQP4 channels. According to them, vascular basement membranes have a crucial role in the maintaining of optimal brain activity.

Schley D *et al.* [20] in their comprehensive study present the analysis of the elimination of ISF and in it dissolved substances, as well as Aβ from the brain grey mass, by pericapillary and periarterial pathways. However, an open question remains about the driving forces of this flow, with the opposite direction in relation to the luminal direction of the bloodstream. Using a mathematical model, the authors determine that arterial wall pulsations are the source of these forces. The drainage occurs through a thin layer of the arterial wall between the VSMCs. During each pulse cycle, there is a period when the ISF and dissolved substances are moved in the opposite direction in relation to the direction of the blood flow in the arterial lumen. According to the authors, successful drainage depends on how and how much of the mentioned substances will be attached to the surface of the draining pathway and achieve the valve-like effect. The decline of the pulse amplitude, typical for old arteries, will prolong the attaching time and consequently speed up Aβ deposition (typical for AD).

Weller RO *et al.* [21] point out that the capillary and arterial basement membranes are an important perivascular pathway by which ISF and in it dissolved substances are eliminated from the brain. According to these authors, their theoretical model shows that arterial pulsations are responsible for effective perivascular drainage of ISF and dissolved substances. During the aging process arteries become more rigid. The amplitudes of their pulsations diminish, the drainage of Aβ declines, and its extracellular aggregation and accumulation in artery walls become marked and harmful. These authors have the same view on the subject as Schley at al. [20].

Albargothy NJ *et al.* [22] emphasize the generally known lack of conventional lymphatic vessels in the brain. They quote researchers who, using high-resolution studies with formalin-fixable fluorescent tracers, have proved that the draining pathways for ISF and in it dissolved waste substances are situated within the basement membranes in the walls of cerebral capillaries, arterioles, and arteries. In cerebral arteries, tracers were located in the basement membranes around VSMCs in the tunica media and were not observed in the basement membranes linked with the endothelium and the pia/glial system (inner and outer basement membranes of VSMCs). The authors of this study, as well as the other mentioned investigators, named this pathway as the intramural periarterial drainage pathway (IPAD). By injecting 2 μL of 100 μM soluble fluorescent fixable Aβ into the CSF of the cisterna magna, the authors

have examined the Aβ particle positions in 6-10 and 24-30 month old male mice brains. Immunocytochemistry and confocal microscopy have shown very quickly (5 min after injecting) the presence of Aβ colocalized with α-2 laminin in the pial-glial basement membranes, in other words, in the paravascular drainage space. 30 min after injecting, Aβ colocalized with collagen IV was found in the smooth muscle cell basement membranes in the media of arterial walls. They have not found signs of drainage along the venous walls. By means of these experiments, they have concluded that the accumulation of Aβ particles in the presented media route is crucial for CAA development. Instead of naming the draining pathway located between the pia and astrocytic endfeet as the paravascular drainage pathway, the authors of this study use the expression pial-glial basement membranes on the outer aspects of the artery wall. In my opinion, both expressions stand for the same thing. The authors have excellently presented the direction of the tracer's movement along the pial-glial basement membrane (paravascular pathway), which has the same direction as the intraarterial blood flow (Fig. 6 in Ref. 22).

Engelhardt B *et al.* [23] also indicate the imperfection of lymphatic vessels in the brain. This group is especially involved in the investigation of anatomical connections of fluid drainage from the CNS up to the regional lymphatic nodes. They are especially interested in the transfer of antigen-presenting cells through this drainage. According to them, these drainage pathways, connected with the narrow basement membrane pathways within the walls of cerebral capillaries and arteries, can not traffic antigen-presenting cells. The authors analyze in detail the contribution of damage to these pathways to the onset of multiple sclerosis and AD. This study also very well distinguishes and presents (Fig. 4 in Ref. 23) both the peri and paravascular drainage pathways.

Many authors consider that a number of possible mechanisms enable the clearance of ISF and Aβ in the direction opposite to the pulse wave, among them the attachment mechanism, effects of flexible structures inside the basilar membranes, and the valvular mechanism. All these mechanisms generate a greater resistance for the forward periarterial flow than for the retrograde periarterial flow which is opposite to the direction of the pulse wave. However, all these proposed mechanisms are not exactly verified. Among these mechanisms, the opinion that the driving forces are induced by VSMCs which are embedded in the fluid-filled poroelastic medium increasingly prevails [20, 24, 25].

It is necessary to give due attention to the study of Diem AK *et al.* [24, 25]. The authors point out that AD is the most frequent form of dementia, and currently there is no effective prevention and therapy for this disease. The decline of cognitive functions in AD correlates with Aβ peptide accumulation inside the walls of capillaries, arterioles, and arteries. The elimination of SF and Aβ takes place along the basement membranes of the mentioned vessels, and this is the IPAD. Using a mathematical model, the authors have proved that arterial pulsations are not strong enough to induce adequate drainage velocities with the opposite direction in relation to the pulse wave and blood flow. They have also proved that a specific valvular mechanism such as directional permeability is necessary for adequate drainage. The driving forces of this flow are not induced by cardiac pulsations. The mentioned authors continuously emphasize that the brain does not have a standard lymphatic circulation.

Basement membranes which constitute the IPAD are built of composite extracellular matrix proteins, where matrix, in fact, is a porous medium through which ISF and fusible metabolites are drained. All the facts show that ISF enters the basement membranes at the capillary level and flows towards the cervical lymph nodes along the basement membranes of cerebral arteries. The opposite direction of the flow can be obtained by the valve mechanism. The attempt to generate the flow model by means of the pressure gradient dependent on permeability has shown that due to the long arterial pulsation wave, the pressure gradient inside the basal membranes has a significantly low value necessary to start the fluid moving. It is concluded that the arterial pulsations are insufficiently strong to move the perivascular drainage inside the basement membranes of cerebral arteries. Applied mathematical models, as well as experimental studies, have refuted the widely accepted hypothesis related to the arterial pulsations as the driving force for Aβ drainage. The mentioned flexible and valve-like structures inside the cerebral artery walls still require an experimental confirmation [24, 25].

It has been emphasized that Diem AK *et al.* [24, 25], after comprehensive experimental and mathematical investigations, have concluded that arterial pulsations are not strong enough to induce sufficient drainage velocities. They consider that the necessary flexible and valvular structures have not been exactly defined, requiring additional comprehensive investigations. Accepting the neurovascular hypothesis as the new working hypothesis, these authors undertake additional analysis of the waste clearance mechanism. The essential point of this hypothesis is the functional hyperemia, *i.e.* the cerebral arteries and arterioles' dilatation as the consequence of the elevated activity of neurons and their greater necessity for nutrient materials, respectively, emergents. According to the authors, any kind of physical or cognitive activity induces increased neuronal activity connected with the significant rise in their necessity for oxygen, glucose and other nutrients. Through the mechanism of neurovascular coupling arteriolar and arterial dilatation occurs. Based on this coupling the authors have presented the neurovascular hypothesis by which they attempted to explain the origin of the driving forces for the drainage of Aβ and waste products along the perivascular pathway (IPAD).

Undertaken investigations by Roxana Aldea R *et al.* [26] represent a great turning-point in the former understanding of the drainage issue and give the most probable solution to the problem. These investigators emphasize that the brain, with a high metabolic rate, does not have its own traditional lymphatic system for waste materials drainage. According to them, this system has been replaced with the basement membranes of capillaries, arterioles, and arteries. They become exclusive carriers of the IPAD. The former widely accepted view about the importance of arterial pulsations for this drainage, according to them, has now been substituted by the opinion that arterial pulsations are not strong enough for this function, and the role of the drainage is taken over by internal, inborn mechanisms of the arterial wall, *i.e.* VSMCs and the vasomotion phenomenon (IPAD).

This periarterial pathway, exceptionally important for waste drainage, especially for Aβ, from the brain up to the cervical lymph nodes, passes along the basement membranes located between the layers of VSMCs in the cerebral artery

media. The flow of waste material, including Aβ, has an opposite direction in relation to the blood flow and pulse wave. The pathway begins with the entrance of waste particles into the capillary basement membranes, passes through their interior proximally and enters into the arterial basement membranes, but not into the outer and inner basement membranes of VSMCs. Forces generated by VSMCs lead to the IPAD, based on the deformation possibilities of arterial media membranes. These cells otherwise generate the basal vascular tone. Their spontaneous rhythmic contractions, called vasomotion, are independent as to arterial pulsations induced by cardiac action and respiratory excursions, and according to the mentioned authors, constitute the basis for the driving forces of V-IPAD (vasomotion driven IPAD). The explanation of these events is as follows: basement membranes are in fact special soft plates internally filled with a poroelastic medium, whose pores open or close depending on the VSMCs' contraction or relaxation. The contraction of two neighboring VSMC layers induces the squeezing of the embedded basement membranes between them, forcing the fluid, filled with Aβ molecules and other waste particles, out from the open pores. The fluid flows further on proximally or retrogradely, following the vasomotion wave. Consequently, the elastic deformation of the basement membranes, induced by the VSMCs' contractions, has a crucial role in the flow regulation along the IPAD pathways. The vasomotion wave has the opposite direction in relation to the blood flow and pulse wave. (***Fig. 3,4***) [26].

The vasomotion problem deserves special attention. According to the generally accepted definition, vasomotion is a spontaneous oscillation of the vascular musculature tension, independent from the heartbeat, innervation or respiration. Its mechanism has not yet been explained. However, it is believed that hormones (aldosterone, angiotensin II, antidiuretic hormone), neuronal factors (baroreflexes, chemoreflex), and local factors (vasodilatation as a response to local ischemia or the local accumulation of waste material) have a certain influence on these events. Some investigators consider that the reason for periodic oscillations is the rhythmic intracellular oscillations of Ca^{2+} ion concentration in the VSMCs. It is considered that the oscillating values of arterial diameter have a more favorable effect on blood flow in comparison to static diameter values. Vasomotion has its own rhythmic frequency usually in the range of 1-2 oscillations per min. The starting point for the vasomotion is related to small arterioles, metarterioles, and precapillary sphincters, and the direction of its manifestation is opposite to the blood flow. However, there is also the opinion that the slow oscillating inflow of blood into the capillaries induces a much better oxygenation of tissues than uniform flow (***Fig. 4, Table 2***). According to a number of recent investigations, it has become evident that Aβ interacts unfavorably with VSMCs in the arterial tunica media, causing their structural cytoskeletal alterations, especially disruptions of actin. The resulting damage in the VSMCs' structure and function, induced primarily by the toxic Aβ effects, lead to the break down of vasomotion and to a consequent reduction in the driving forces for the perivascular drainage of Aβ and other waste elements. It seems that the aging process also has an unfavorable effect on this drainage [21, 26-34].

Raffaello A *et al.*[27] have explained some facts about the role of Ca^{2+} ions connected with the vasomotion phenomena. The mentioned intracellular Ca^{2+} concentration oscillations are the result of its periodical release from stores located in the VSMCs' endoplasmic reticulum (ER). The Ca^{2+} release is enabled by the activation of special Ca^{2+} channels, *i.e.* receptors (InsP3R, inositol trisphosphate receptor) located at the ER membrane. The activator is the soluble inositol phosphate signaling molecule (InsP3, IP3 [inositol trisphosphate]) which has been generated by the phospholipase C (PLC) hydrolysis of the cell membrane phospholipid (PIP2, phosphatidyl inositol 4,5-bisphosphate). After the binding of IP3 to InsP3R, Ca^{2+} is released into the intracellular space with the consequent activation of the contractile mechanism. Two types of reactions have been observed: either Ca^{2+} "sparks" located strictly on one point of the ER membrane, or traveling Ca^{2+} exits from ER in the form of waves which move along the complete cell interior. The strong Ca^{2+} exit from ER during the InsP3R stimulation by InsP3 elevates its cytosolic concentration up to a specific threshold, and after this, every tendency for elevation induces the inhibition of InsP3R and Ca^{2+} decrease. Cytosolic Ca^{2+} oscillatory elevations are responses to agonist-induced InsP3 release. The Ca^{2+} being restored from the cytosol to ER is feasible by the sarco(endo) plasmic reticulum calcium ATPase (SERCA) located on the ER membrane. Arteriolar and arterial vasomotion waves occur in the case when there exists good synchronization between the great number of individual wave oscillations (***Fig. 5***). (For the complete explanation of these events, the so-called crosscoupling hypothesis has been used - see Fig. 1. in Ref. 27).

Gaspers LD *et al.*[35] in their paper also have emphasized, like Raffaello [27] and her group, the fundamental role of inositol 1,4,5-trisphosphate (InsP3, IP3) in generating Ca^{2+} cytosolic oscillations and waves. At the beginning of their presentation, the authors analyze in detail the VSMC plasma membrane and on it located several important proteins. They are the G protein-coupled receptor (GPCR, seven-transmembrane receptor), phosphatidylinositol 4,5-bisphosphate (PIP2) and phospholipase C (PLC). Activated by Ca^{2+} ions (which have arrived in the cytosol from ER), PLC hydrolyzes PIP2 into two second messengers: IP3 and diacylglycerol (DAG). DAG remains further in the membrane, and fusible IP3 diffuses up to the ER membrane and attaches to InsP3R (InsP3 receptor, *i.e.* Ca^{2+} channel) which is when strongly activated by IP3, responsible for the Ca^{2+} exit from the ER. After the exit into the cytosol, Ca^{2+} binds to specific contractile elements inducing strong VSMC contraction. On account of the stronger exit of Ca^{2+} from the ER, its concentration in the cytosol rises. This leads to PLC and IP3R stimulation, but with the approach of the Ca^{2+} concentration up to the specific threshold, the situation changes, and now the elevated Ca^{2+} blocks IP3R and the Ca^{2+} exit into the cytosol. PLC activation also drops, as well as the frequency and strength of Ca^{2+} waves eventually with their complete disappearance. Now, a new activation of the complete cycle starts and continues so further on (permanent activation of GPCR by ligands). This mechanism produces intracellular Ca^{2+} oscillations accompanied with its traveling waves and the consequent vasomotion. The detailed description of these biochemical and physiological events, however, exceeds the boundaries of my paper (***Fig. 5***). (For a better understanding of Gaspers LD *et al.* Investigation [35], the reader can analyze the Graphical Abstract – Effects of IP3 buffering on Ca^{2+} oscillations at the beginning of their paper).

It is also necessary to explain some facts visible on the left upper side of ***Fig. 5***. The two functional states of the myosin-actin interaction, smooth muscle contraction and relaxation, can be recognized. Presented are actin and myosin filaments; myosin heavy and light chains; calmodulin, which can be free or connected with Ca^{2+}; MLCK (myosin light chain kinase) and pp60SRC kinases. The role of myosin regulatory light chain (RLC) is especially emphasized. On the lower part of ***Fig. 5***, presented are events connected with Ca^{2+} binding with calmodulin, MLCK phosphorylation and activation, phosphorylation of the RLC, cross-bridge formation between actin and myosin, as well as the SMCs contraction and vasomotion generation. Relaxation occurs after Ca^{2+} removal from the cytosol, especially by CaATPases, which throw Ca^{2+} out of the cell into the ECS and back into the ER. MLCK inactivation occurs after Ca^{2+} dissociation from calmodulin (CaM). Myosin dephosphorylation occurs by the action of type 1 protein serine/threonine phosphatase (MLCP) (***Fig. 5***)[36, 37].

All the mentioned views and existing theories require further intensive investigations.

The iron ion pathways in the brain represent an important area of scientific exploration. The overexpressed Fe^{2+} must be drained. Through AQP4 channels and intercellular astrocytic endfeet clefts, Fe^{2+} from ECS enters into the paravascular space, passes through it, and across the markedly permeable pia enters into the subarachnoid space (SAS, CSF). Through arachnoid granulations, a part of Fe^{2+} enters into the venous blood of the sagittal superior sinus and further drains into the systematic circulation. A portion of Fe^{2+} is reabsorbed into the blood by BCSFB and divalent metal transporter (DMT1) and is drained further on by the venous flow. The driving forces of this pathway have been explained in the earlier parts of the text[5, 38].

As mentioned before, all experimental findings, as well as laboratory analyses, indicate that AD is closely connected with Aβ aggregate accumulation; metal dyshomeostasis, especially related to iron; and oxidative stress. It can be concluded that blocking aggregation as early as possible, and the elimination of iron ions, especially Fe^{3+} ions, could slow down the pathologic reduction into Fe^{2+}, as well as its oxidation in the Fenton reaction ($Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{-}OH + {}^{*}OH$) with the formation of toxic and aggressive *OH (hydroxyl radical).

Table 2. Essential characteristics of the vasomotion (perivascular drainage).

Different parameters	Description of events
Starting point of the vasomotion wave	Cerebral arterioles
Direction of the wave	Retrograde in relation to the blood flow course
Cause of the wave	Vascular smooth muscle cell contraction and relaxation
Squeezing of the VSMCs in basement membranes	Squeezing of the VSMCs BB between two neighboring VSMCs layers; closing the poroelastic pores of the VSMCs basement membranes; exit of the Aβ deposited molec. and waste particles from pores
Direction of Aβ and waste flow in the compressed intermedial fluid	Retrograde in relation to the arterial blood flow and identical to the vasomotion wave direction
Different possible stimuli for vasomotion start	Arterial blood pressure, shear stress, neuronal metabolic activity, several types of innervation, Ca^{2+} involvement
Medium of Aβ and waste flow propagation: vasomotion wave is also spread through the same medium	Arteriolar and arterial media with its VSMC basement membranes; the inner and outer VSMC basement membranes are excluded

The table is composed especially related to Ref. 24, Ref. 25, and Ref. 26; in the future, the presented data should be examined, evaluated and supplemented in detail.

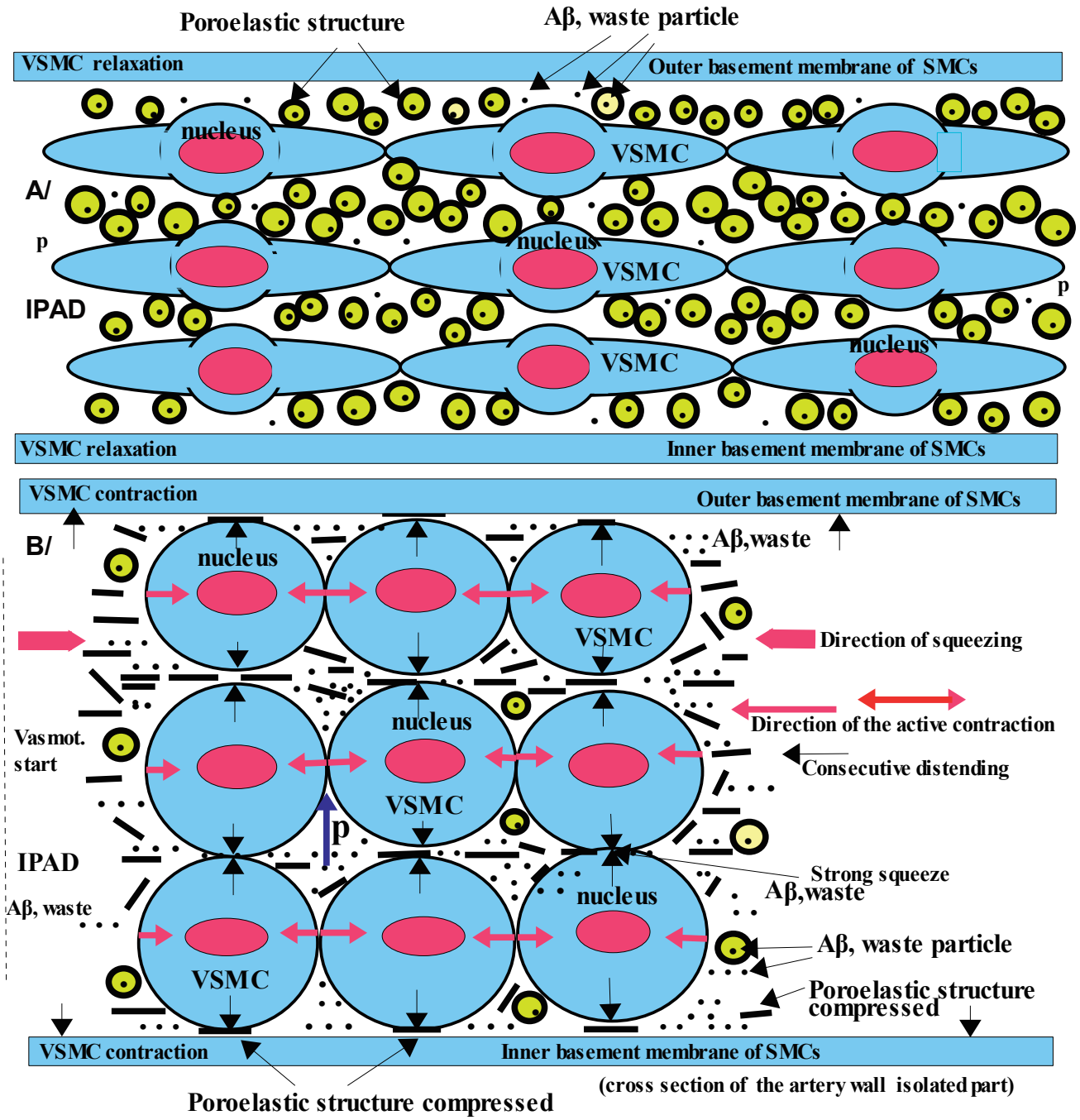

Fig. 3. **Schematic presentation of VSMC contraction and relaxation.**

The two states of the vasomotion wave, relaxation and contraction. Both states are dependent upon the complex processes linked with a set of interrelated intracellular proteins in VSMCs. It is too complicated to enter into a detailed analysis of these processes. **A**/ small circles with a black point in their center represent pores in the poroelastic medium between the layers of VSMCs. Pores in the relaxed state are places of fluid, Aβ, and waste accumulation. In fact, this medium presents the VSMC basement membranes. **B**/ VSMC contraction induces a strong elevation of the pressure between cells, compression of pores, and the squeezing out of fluid and its components. According to the start point of the vasomotion wave and its retrograde direction, the induced intermedial pressure gradient drives the fluid and dissolved substances also in the retrograde direction; IPAD, intra-mural peri-arterial drainage; VD-IPAD, vasomotion-driven IPAD; inner basement membrane of SMCSs, the layer of connective tissue tightly connected with endothelium; outer basement membrane of VSMCs, the layer of connective tissue tightly connected with the vessel pia, the space between the VSMCs layers is a complex composed of smooth muscle cell basement membranes (VSMCs, media, IPAD); VSMCs, vascular smooth muscle cells; Aβ, amyloid-β; Vasmot,, vasomotion.

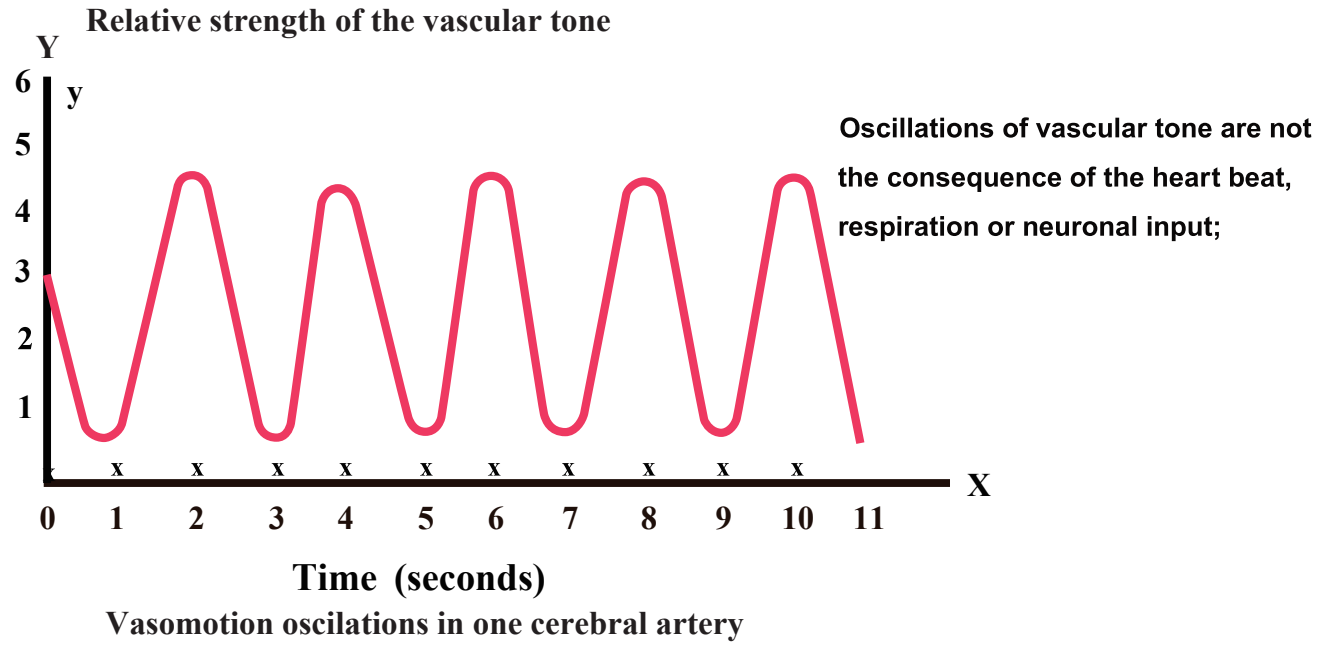

Fig. 4. **Schematic presentation of the vasomotion waves.**

The orientation vasomotion curve was made according to several findings in the available literature (Ref. 26, Ref. 27, Ref. 31). It can be concluded that the frequency of the vascular tension oscillations (vasomotion) of the majority of body arteries is in the range of 1-20/min. It is also observed that the oscillation strengths are not uneven. Along with occurrence *in vivo* oscillations appear also in vitro. According to Ref. 31, there are three models which can explain the origin of oscillations. The first model shows the importance of the oscillating liberation of Ca^{2+} ions from the intracellular stores (cytosolic oscillator). The second model shows the oscillation generation in the sarcolemma (a membrane oscillator). The third model is based on glycolysis oscillations (metabolic oscillator). The most investigated was the first model. The starting point for vasomotion is related to small arterioles, metarterioles, and precapillary sphincters. The direction of the vasomotion waves is retrograde, opposite to the pulse wave and to the direction of the blood flow. Based on the interchanged contractions and relaxations of the smooth muscles of arterioles, metarterioles, and precapillary sphincters (called vasomotion, angiokinesis), the blood flows along capillaries in single moments, and not continuously. However, future investigations have the role to explain the exact cause of this phenomenon.

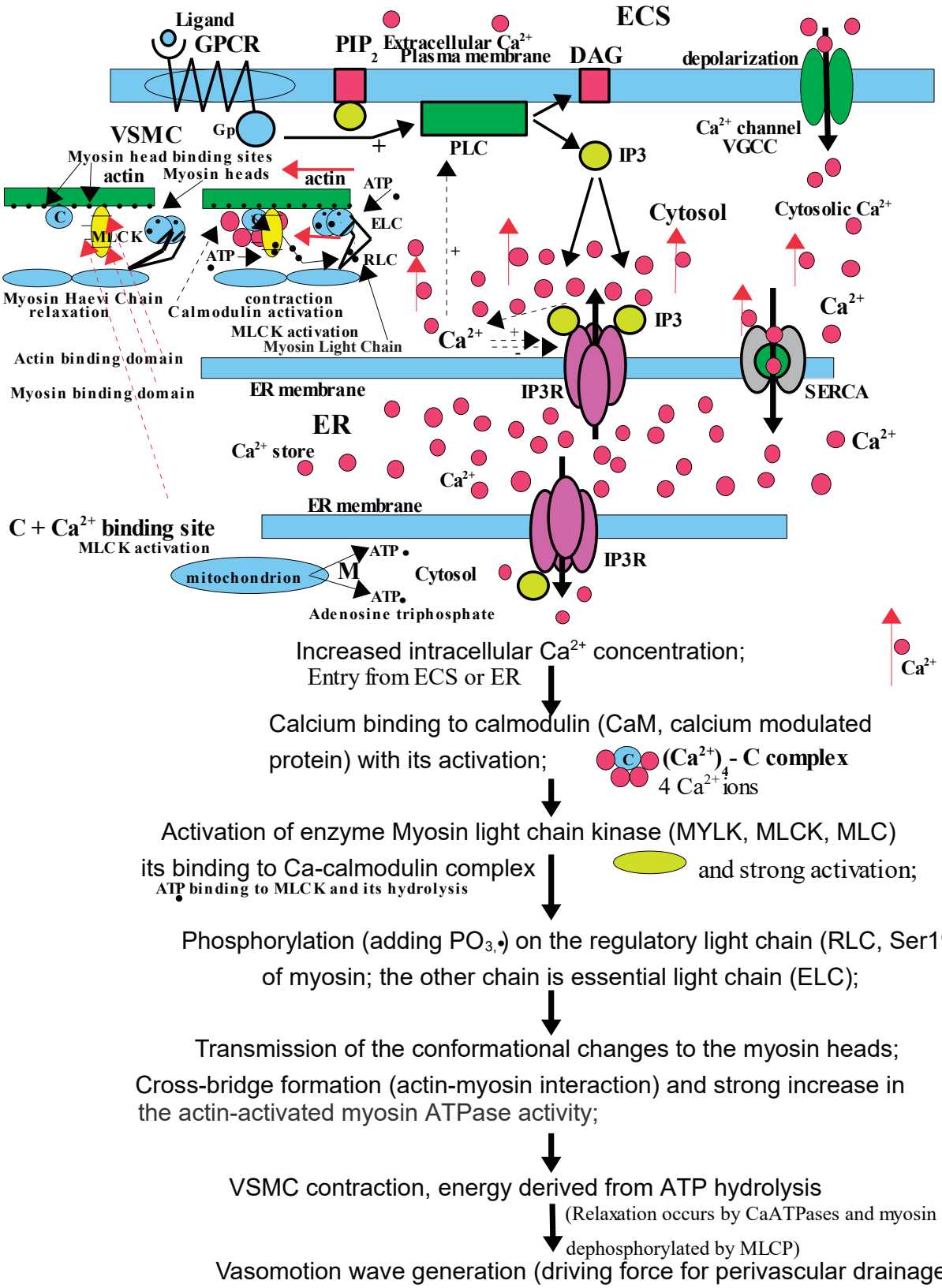

Fig. 5. **Explanation of VSMC contraction and vasomotion generation.**

VSMC, vascular smooth muscle cell; GPCR, G-protein-coupled receptor; PLC, phospholipase C; PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; InsP3, inositol 1,4,5-trisphosphate; ER, endoplasmic reticulum; SERCA, serca(endo)plasmic reticulum calcium ATPase; ATP, adenosine triphospahte; ECS, extracellular space, cytosol; Ca^{2+}, calcium ion; VGCC, voltage-gated calcium channel; •, PO3 , phosphate and ATP-binding site; •, actin-binding site; myosin ATPase enzyme; M, mitochondria; RLC, regulatory light chain; ELC, essential light chain; Gp, G-protein; C, calmodulin; IP3, inositol trisphosphate; IP3R, IP3 receptor; MLC, myosin light chain; MLCK, MLC kinase; MYLK, myosin light chain kinase: CaM, calmodulin.

Contemporary therapy of Alzheimer's disease (AD)

Currently, two groups of drugs are in extensive use: cholinesterase inhibitors and antagonists of N-methyl-D-aspartate (NMDA) receptors. The first group includes donezepil hydrochloride (Aricept), rivastigmine (Exelon), and galantamine hydrobromide (Reminyl). They inhibit the action of enzyme acetylcholinesterase (AChE) which catalyzes the breakdown of acetylcholine (ACh) that functions as a neurotransmitter. The second group includes memantine (Ebixa) which by binding to NMDA receptors (NMDAR, N-methyl-D-aspartate receptor, glutamate receptor) protects the brain cells from the harmful effects of, in AD, elevated concentrations of glutamate released from damaged brain cells [1, 6, 7, 39].

Among a number of therapeutic attempts, it is important to emphasize the growing interest in chelation therapy, chelators, and nanoparticles. The most significant is Curcumin (Diferuloymethane) and its derivatives with their strong affinity for Fe^{3+} which they catch, bind, and by means of nanoparticles effectively drain out from the brain (chelator effect). In the binding and draining of one Fe^{3+}, three curcumin molecules are included [39]. The similar favorable effect is also found in the main component of green tea polyphenol (epigallocatechin-3-gallate, EGCG). Bound with nanoparticles, this compound presents an evidently strong chelator [39]. Another important chelator is desferrioxamine B (DFOB), a hexadentate chelator which strongly binds Fe^{3+} and the somewhat weaker Al, Zn, and Cu, therefore it slows down the course of AD [39]. Intracellular and extracellular Aβ degradation presents the next therapeutic approach for AD. For this purpose, proteasomes are used, which operate by the ubiquitin-proteasome pathway. During this type of degradation lysosomal cathepsin enzymes and thiolmetalloendo peptidases are especially prominent. Connected with extracellular Aβ degradation in all cases, it is necessary to mention Neprilysin (a membrane-anchored zinc metalloendopeptidase), matrix metalloproteinases 2, 3 and 9, as well as glutamate carboxypeptidase II 7 [7, 39].

Among other therapeutic approaches directed to Aβ reduction, special emphasis is given to immunotherapy which includes active vaccination and passive immunization. Xiang Y *et. al.* [40] are especially interested in the effects of peripheral tissues in the clearance process of Aβ produced in the brain. These authors have proved that Aβ is degraded and cleared during its passing through the capillary system of the liver, kidneys, gastrointestinal tract, and skin. The damage to this periphery pathway leads to evident deterioration of the course of AD. The Apolipoprotein E (ApoE) treatment has a beneficial effect on these events.

It is necessary to present some recent data about AD therapy using ApoEε2 isomer direct intracranial application by means of a special stereotaxic instrument, or by the intravenous administration of the biotinylated polyamides conjugated to streptavidin-conjugated ApoEε2. This complex can pass across the BBB by interaction with the ApoE receptor located in this barrier. All these methods are characterized by the interaction between the Aβ and ApoEε2, their binding, and the complete removal of the formed complex out from the brain [7, 40-43].

In the end, it is also important to emphasize a number of other therapeutics which are included in the attempts to achieve some beneficial effects for AD therapy. They are antioxidants, AGE breakers, RAGE blockers, and antiglycation compounds. There are also some attempts of treatment with alagebrium (ALT-711), aminoguanidine, 4,5-dimethyl-3-phenacylthiazolium chloride (DPTC), benfotiamine, thiamine, and pyridoxamine. However, the results of these treatments are questionable [1].

Presently, the general opinion is that chelation therapy is the most effective approach for AD course retardation.

Conclusion

AD is a chronic, progressive, wasting neurodegenerative disease, presently still with a mostly unknown etiology. Connected with age and the aging process, it shows, due to the great absolute and relative increase of the worldwide elderly population, a growing incidence and prevalence. The costs for its prevention, therapy, and social care have an exponential rise. In its pathology, there are two most important events: first, the intracerebral accumulation of the amyloid beta peptide, generated by proteolysis of APP which is present in neuronal membranes; and the second, the extracellular accumulation of metal ions, especially iron ions, and accompanying oxidative stress. In this respect, the appropriate elimination of intracerebral Aβ peptides and elevated iron ion concentration has become most essential. The elimination pathways are being increasingly investigated as well as the moving forces of the draining mechanism. A better understanding of these pathways and the mentioned moving forces gives hope for more effective prevention and therapy of this serious lethal disease in the near future.

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Conflict of interest

The authors declare no conflict of interest in this study.

References

1) Barić N. Role of advanced glycation end products in Alzheimer's disease. Glycative Stress Res. 2014; 1: 68-83.
2) Barić N. Role of advanced glycation end products (AGEs) on the reactive oxygen species (ROS) generation in Alzheimer's disease amyloid plaque. Glycative Stress Res. 2015; 2: 140-155.
3) Viles JH. Metal ions and amyloid fiber formation in neurodegenerative diseases. Copper, zinc and iron in Alzheimer's, Parkinson's and prion diseases. Coordination Chemistry Reviews. 2012; 256; 2271-2284.
4) Maynard CJ, Bush AI, Masters CL, et al. Metals and amyloid-β in Alzheimer's disease. Int J Exp Pathol. 2005; 86: 147-159.
5) Oshiro S, Morioka MS, Kikuchi M. Dysregulation of iron metabolism in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Adv Pharmacol Sci. 2011; 2011: 378278.
6) Barić N, Cetina M. Initial bonding of ferric ion (Fe^{3+}) and amyloid beta (Aß) peptide as the precondition for oxidative stress in Alzheimer's disease. Glycative Stress Res. 2017; 4: 250-265.
7) Barić N. Increase in iron intracerebral concentration in patients suffering from Alzheimer's disease follows the rise of amyloid beta. Glycative Stress Res. 2019; 6: 7-20.
8) Page CC, Moser CC, Chen XX, et al. Natural engineering principles of electron tunneling in biological oxidation-reduction. Nature. 1999; 402(6757): 47-52.
9) Ayton S, Faux NG, Bush AI. Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. Nat Commun. 2015; 6: 6760.
10) Roth AD, Ramirez G, Alarcón R. et al. Oligodendrocytes damage in Alzheimer's disease: Beta amyloid toxicity and inflammation. Biol Res. 2005; 38: 381-387.
11) Xu J, Chen S, Ahmed SH, et al. Amyloid-beta peptides are cytotoxic to oligodendrocytes. J Neurosci. 2001; 21: RC118.
12) Cai Z, Xiao M. Oligodendrocytes and Alzheimer's disease. The International Journal of Neuroscience. 2016; 126: 97-104.
13) Wawrzyniak-Gacek A, Łuszczewska-Sierakowska I, Matysek M. Histochemical evaluation of iron content in oligodendrocytes in selected regions of the rat brain. Arch Physiother Glob Res. 2014; 18: 39-42.
14) Bedussi B, Almasian M, deVos J. Paravascular spaces at the brain surface: Low resistance pathways for cerebrospinal fluid flow. Journal of Cerebral Blood flow & Metabolism. 2018; 38: 719-726.
15) Brinker T, Stopa E, Morrison J, et al. A new look at cerebrospinal fluid circulation. Fluids Barriers CNS. 2014; 11: 10.
16) Iliff JJ, Wang M, Liao Y. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Science Translational Medicine; 2012; 4: 147 ra111
17) Iliff JJ, Lee H, Yu M, et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. J Clin Invest. 2013; 123: 1299-1309.
18) Bacynski A, Xu M, Wang W, et al. The paravascular pathway for brain waste clearance: Current understanding, significance and controversy. Front Neuroanat. 2017; 11: 101.
19) Morris AWJ, MacGregor Sharp M, Albargothy NJ, et al. Vascular basement membranes as pathways for passage of fluid into and out of the brain. Acta Neuropathol. 2016; 131: 725-736.
20) Schley D, Carare-Nnadi, Please CP, et al. Mechanisms to explain the reverse perivascular transport of solutes out of the brain. J Theor Biol. 2006; 238: 962-974.
21) Weller RO, Subash M, Preston SD, et al. Perivascular drainage of amyloid-β peptide from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. Brain Pathol. 2008; 18: 253-266.
22) Albargothy NJ, Johnston DA, MacGregor-Sharp M, et al. Convective influx/glymphatic system: Tracers injected into the CSF enter and leave the brain along separate periarterial basement membrane pathways. Acta Neuropathol. 2018; 136: 139-152.
23) Engelhardt B, Carare RO, Bechmann I, et al. Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol. 2016; 132: 317-338.
24) Diem AK, MacGregor Sharp M, Gatherer M, et al. Arterial pulsations cannot drive intramural periarterial drainage: Significance for Aβ drainage. Front Neurosci. 2017; 11: 475.
25) Diem AK, Carare RO, Weller RO, et al. A control mechanism for intra-mural peri-arterial drainage via astrocytes: How neuronal activity could improve waste clearance from the brain. PLoS One. 2018; 13: e0205276.
26) Aldea R, Weller RO, Wilcock DM, et al. Cerebrovascular smooth muscle cells as the drivers of intramural periarterial drainage of the brain. Front Aging Neurosci. 2019; 11: 1.
27) Raffaello A, Mammucari C, Gherardi G, et al. Calcium at the center of cell signaling: Interplay between endoplasmic reticulum, mitochondria, and lysosomes. Trends Biochem Sci. 2016; 41: 1035-1049.
28) Di Marco LY, Farkas E, Martin C, et al. Is vasomotion in cerebral arteries impaired in Alzheimer's disease? J Alzheimers Dis. 2015; 46: 35-53.
29) Aalkjær C, Boedtkjer D, Matchkov V. Vasomotion: What is currently thought? Acta Physiol (Oxf). 2011; 202: 253-269.
30) Pradhan RK, Chakravarthy VS. Informational dynamics of vasomotion in microvascular networks: A review. Acta Physiol (Oxf). 2011; 201: 193-218.
31) Bakker ENTP, Bacskai BJ, Arbel-Ornath M. Lymphatic clearance of the brain: Perivascular, paravascular and significance for neurodegenerative diseases. Cell Mol Neurobiol. 2016; 36: 181-194.
32) Aalkjær C, Nilsson H. Vasomotion: Cellular background for the oscillator and for the synchronization of smooth muscle cells. Br J Pharmacol. 2005; 144: 605-616.
33) Kapela A, Nagaraja S, Parikh J, et al. Modeling Ca^{2+} signaling in the microcirculation: Intercellular communication and vasoreactivity. Crit Rev Biomed Eng. 2011; 39: 435-460.

34) Berridge MJ, Galione A. Cytosolic calcium oscillator. FASEB J. 1988; 2: 3074-3082.
35) Gaspers LD, Bartlett PJ, Politi A, et al. Hormone-induced calcium oscillations depend on cross-coupling with inositol 1,4,5-trisphosphate oscillations. Cell Rep. 2014; 9: 1209-1218.
36) Walsh MP. Vascular smooth muscle myosin light chain diphosphorylation: Mechanism, function, and pathological implications. IUBMB Life. 2011; 63: 987-1000.
37) Eddinger TJ, Meer DP. Myosin II isoforms in smooth muscle: Heterogeneity and function. Am J Physiol Cell Physiol. 2007; 293: C493-508.
38) Zheng W, Monnot AD. Regulation of brain iron and cooper homeostasis by brain barrier systems: Implication in neurodegenerative diseases. Pharmacol Ther. 2012; 133: 177-188.
39) Barić N. Chelation therapy for Alzheimer's disease: Nanoparticles as new components of this therapy. Glycative Stress Res. 2018; 5: 104-118.
40) Xiang Y, Bu XL, Liu YH, et al. Physiological amyloid-beta clearance in the periphery and its therapeutic potential for Alzheimer's disease. Acta Neuropathol. 2015; 130: 487-499.
41) Liu CC, Kanekiyo T, Xu H, et al. Apolipoprotein E and Alzheimer disease: Risk, mechanisms, and therapy. Nat Rev Neurol. 2013; 9: 106-118.
42) Wu L, Zhao L. ApoE2 and Alzheimer's disease: Time to take a closer look. Neural Regen Res. 2016; 11: 412-413.
43) Wang X, Li R, Zacharek A. Administration of downstream ApoE attenuates the adverse effect of brain ABCA1 deficiency on stroke. Int J Mol Sci. 2018; 19(11).