钙敏感受体在阿尔茨海默病中作用机制的研究

冯晨希[1] 王媛媛[1] 鲍晓明[2] 沈苏燕[1] 王钦文[1] 徐淑君[1]*

([1]宁波大学医学院, 浙江省病理生理学重点实验室, 宁波 315211; [2]宁波市第二医院, 宁波 315010)

摘要 阿尔茨海默病(Alzheimer's disease, AD)是老年人常见的神经退行性疾病, 目前仍缺乏对其发病机制的深入理解以及有效药物的开发。钙敏感受体(calcium-sensing receptor, CaSR)广泛存在于人体中枢神经系统的各类细胞中, 可溶性β-淀粉样蛋白(β-amyloid protein, Aβ)是CaSR的正构调节剂之一。CaSR参与Aβ的级联放大, 介导Aβ引起的炎性因子的释放以及血管内皮细胞生长因子-A(vascular endothelial growth factor-A, VEGF-A)的过量产生。该文主要对CaSR在AD发病中的作用和机制进行综述, 并对CaSR变构抑制剂在AD治疗中的可能作用进行了展望。

关键词 阿尔茨海默病; 钙敏感受体; β-淀粉样蛋白

The Underlying Mechanism of Calcium-Sensing Receptor in Alzheimer's Disease

Feng Chenxi[1], Wang Yuanyuan[1], Bao Xiaoming[2], Shen Suyan[1], Wang Qinwen[1], Xu Shujun[1]*

([1]*School of Medicine, Ningbo University, Zhejiang Provincial Key Laboratory of Pathophysiology, Ningbo 315211, China*; [2]*Ningbo No.2 Hospital, Ningbo 315010, China*)

Abstract Alzheimer's disease (AD) is a common neurodegenerative disease in the elderly. Nowadays the pathogenesis of AD is unclear. There is still lack of effective drugs for this disease. Calcium-sensing receptor (CaSR) has been found in different kinds of cells in the central nervous system. Soluble β-amyloid protein (Aβ) is one of the positive allosteric modulators of CaSR. CaSR is involved in cascade amplification of Aβ, inflammatory factors and vascular endothelial growth factor-A (VEGF-A) which is caused by Aβ. In this review, we summarized the roles and mechanisms of CaSR in the pathogenesis of AD. CaSR allosteric inhibitors are expected to be potential drugs for AD in the near future.

Keywords Alzheimer's disease; calcium-sensing receptor; β-amyloid protein

阿尔茨海默病(Alzheimer's disease, AD)是老年人中最常见的神经退行性疾病之一, 临床表现有认知功能障碍、语言功能障碍等。到2012年为止, 美国65岁以上的老年人中有520万人被诊断为AD, 治疗费用每年高达2 000亿美元[1]。同时, 我国的AD患者人数已达700万, 预计在2030年将达到1 200万。AD分为散发性AD(sporadic AD, SAD)与家族性AD(familial AD, FAD), 后者又分为早发型AD(early-onset AD, EOAD)与迟发型AD(late-onset AD, LOAD)两种。10%的AD患者是家族性AD, 携带明显的致病基因。家族性AD中LOAD(65岁以后患病)多于EOAD(50~60岁患病)[2-3]。AD的临床治疗药物主要是胆碱酯酶抑制和N-甲基-D-天冬氨酸受体(N-methyl-D-aspartic acid receptor, NMDA)拮抗剂等,

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*通讯作者。Tel: 0574-87609594, E-mail: xushujun@nbu.edu.cn

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*Corresponding author. Tel: +86-574-87609594, E-mail: xushujun@nbu.edu.cn

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但是这些药物只能部分地改善和延缓病情, 并不能从根本上防止和逆转AD的病程[4]。有效药物的开发依赖对AD发病机制的进一步研究。

目前, AD的病因学和发病机制依然不清楚, 主要有β-淀粉样蛋白(β-amyloid protein, Aβ)学说、胆碱能学说、炎症机制、激素因素、血脂改变以及兴奋性神经递质学说等[1,4-6]。由于β-淀粉样蛋白与其他因素的广泛联系, 因此, Aβ学说备受关注。该假说认为, Aβ在脑内聚集、纤维化、沉积并形成老年斑, 是导致AD发病的重要病理生理学基础。Aβ会引起树突棘数目下降、抑制长时程增强(long-term potentiation, LTP)的产生、引起AD模型小鼠早期的认知功能下降[7-8]。AD病人脑脊液中Aβ寡聚体含量与简易智能量表分值相关, Aβ寡聚体含量反应了疾病的严重程度, 脑脊液中Aβ寡聚体可以作为AD的诊断标志物[9]。Aβ的级联放大是导致LOAD的重要原因。

钙敏感受体(calcium-sensing receptor, CaSR)是G蛋白偶联受体(G protein-coupled receptors, GPCRs) C家族的成员, 是一种七次跨膜蛋白。CaSR由胞外结构域(extracellular domain, ECD)、跨膜结构域(transmembrane domains, TMD)和携带一条羧基尾巴的胞内结构域(intracellular domain, ICD)构成。钙敏感受体通常以同二聚体的形式发挥作用, 这种二聚体的形成依靠胞外区域中相邻的两个片状的捕虫夹结构域(venus-flytrap, VFT)之间形成的共价或者非共价结构[10]。CaSR内源性的调节剂(正构调节剂)包括Ca^{2+}、一些二价或三价阳离子(Be^{2+}、Sr^{2+}、Mg^{2+}、Gd^{3+}、La^{3+}、Ba^{2+})、多氨等。变构调节剂包括激活CaSR的拟钙剂(calcimimetics)和抑制CaSR活性的钙溶解剂(calcilytics)(表1)。有意思的是, Aβ也是CaSR的正变构调节剂之一。通过邻位对接技术, Aβ和CaSR已经被证明两者之间有直接相互作用[10-11]。钙离子、Aβ等配体能与ECD和TMD上的相应位点结合, 引起TMD和ICD发生构象变化, 通过ICD的C-端与各种G蛋白相互作用, 进而介导下游信号通路的活化或抑制[10-11]。

钙敏感受体除了在外周器官(如甲状旁腺、胃肠道、心血管、骨骼、肾等)分布外, 在中枢神经系统中也有广泛的分布并执行着不同的生理功能(表2)。生理情况下, CaSR主要在神经元与少突胶质细胞表达。在丘脑、下丘脑等脑区的灰质区域和海马CA2区域都高度表达CaSR。下丘脑促性腺激素释放激素细胞和垂体黑素细胞刺激激素分泌细胞中的CaSR分别参与促性腺激素释放激素和促黑色素细胞刺激激素的产生和释放[12-13]。交感神经节神经元和海马锥体神经元中的CaSR在大脑发育过程中促进轴突生长和树突分枝[14]; CaSR参与调节海马神经元中非选择性阳离子通道(non-selective cation channel, NSCC)和Ca^{2+}激活的K^+通道(Ca^{2+}-activated K^+ channels, CACK)的开放, 阻碍可塑性的形成和神经递质的交换[15]。对皮层神经元的研究同样发现, CaSR能阻断NSCC的传导, 抑制兴奋性突触后电流(excitatory post-synaptic currents, EPSC)的振幅[16]。通过对小脑的白质区域CaSR表达量的检测发现, 少突胶质细胞中CaSR增加时间与脑中髓鞘形成期一致[17], 少突胶质细胞中的CaSR通过促进Ca^{2+}激活的K^+通道开放, 诱导神经干细胞加速分化为少突胶质细胞, 从而维护中枢神经系统离子微环境的稳定和调节少突胶质细胞的发育和功能[18]。小鼠星型胶质细胞中, CaSR的表达量相对神经元要低[19], 在病理刺激下, 例如缺血、缺氧的条件下, 在小鼠反应性星型胶质细胞中可以检测到CaSR的表达量增加[20]。而无论生理或病理情况下, 小胶质细胞内CaSR含量都很低或检测不到[19]。

CaSR在中枢神经系统中起了重要的生理作用, 参与调节神经系统中的激素的释放和胞内钙稳定, 参与突触的传递和可塑性的调节。生理情况下,

表1 钙敏感受体的不同配体

Table 1 Different ligands of calcium-sensing receptors

配体种类 Ligand types	举例 Examples
Ca^{2+} and other multivalent cations	Ca^{2+}, Mg^{2+}, Sr^{2+}, La^{3+}, Gd^{3+}
Peptides	β-amyloid protein, polylysine, polyarginine, spermine, spermidine, protamine,
L-amino acids	L-tryptophan, L-histidine, L-phenylalanine, L-valine
Aminoglycoside antibiotics	Tobramycin, neomycin, gentamicin
Calcimimetics	NPS R-568, cinacalcet hydrochloride
Calcilytics	NPS 89636, NPS 2143

表2 CaSR在不同的中枢器官和神经细胞中的作用

Table 2 The role of CaSR in different central organs and nerve cells

器官和细胞 Organs and cells	生理功能 Physiological functions	参考文献 References
Gonadotropin-releasing hormone (GnRH) neuronal cell and melanocyte stimulating hormone (MSH)-secreting cells	Production and release of gonadotropin-releasing hormone and melanocyte-stimulating hormone respectively	[12-13]
Perinatal mouse sympathetic ganglion neurons and hippocampal pyramidal neurons	Promote the growth of neural processes	[14]
Hippocampal pyramidal neurons from wild-type and CaSR-deficient mice	Hinder synaptic response and plasticity	[15]
Cortical terminals, cortical neuronal pairs and isolated neuronal soma	Reduce synaptic transmission	[16]
Immature rat oligodendrocytes	Maintain the CNS ionic microenvironment and regulate oligodendroglial development and function	[17-18]

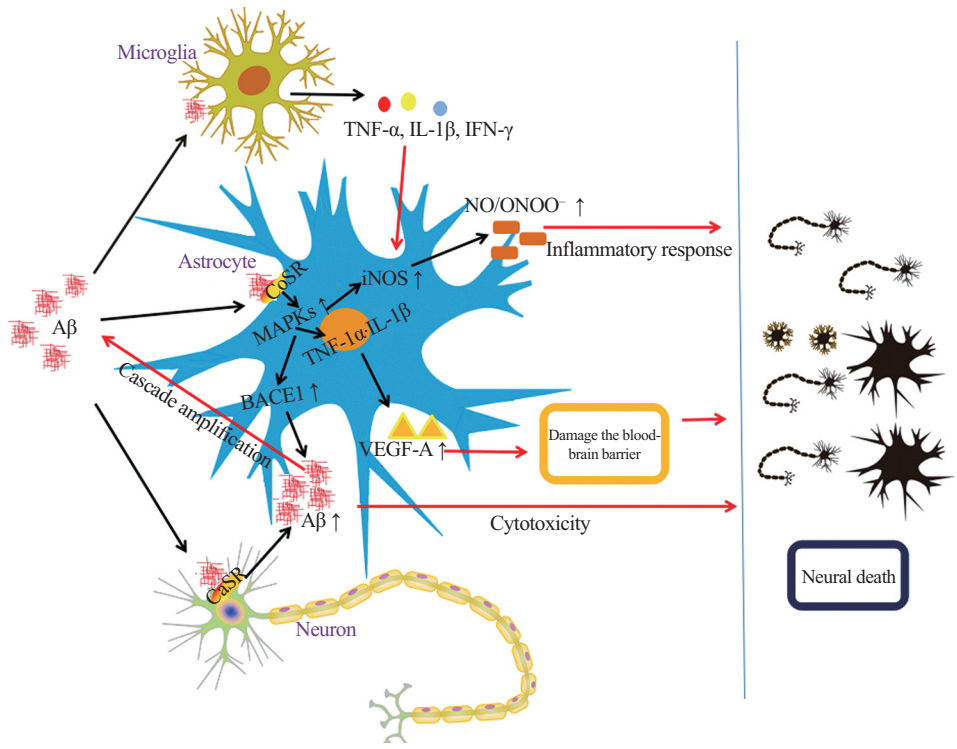
CaSR并没有被完全激活[12,16]。AD时, Aβ产生增加, CaSR被Aβ激活从而介导一系列的病理过程[10-11]。研究表明, *CaSR*基因4号内含子内(CA)n二核苷酸多态性以及单倍体的非同义单核苷酸多态性与AD相关[21]。CaSR参与Aβ的级联放大, 介导Aβ引起的多种毒性因子的释放[22-23](图1)。CaSR在AD发病中的作用、机制以及其变构抑制性药物在AD治疗中的潜在作用引起了研究者的兴趣。

1 CaSR参与Aβ的级联放大

研究表明, 微量的$A\beta_{25-35}$就能有效地促进正常成年人星形胶质细胞(normal adult human astrocytes, NAHAs)中内源性$A\beta_{1-42}$寡聚体的生成和释放。星形胶质细胞中, CaSR介导丝裂原活化蛋白激酶(mitogen-activated protein kinases, MAPKs)信号通路的激活, 诱导了β切割酶1(beta-site APP-cleaving enzyme 1, BACE1)的表达, 导致$A\beta_{1-42}$产生增多[24]。CaSR的变构激动剂NPS R-568能显著地增加星形胶质细胞中内源性$A\beta_{1-42}$的释放。CaSR的变构抑制剂NPS 2143预处理后能完全抑制$A\beta_{25-35}$诱导的内源性$A\beta_{1-42}$寡聚体的增加, 使细胞内$A\beta_{1-42}/A\beta_{1-40}$比值保持正常水平[25-26]。NPS 2143预处理的星形胶质细胞可以使CaSR蛋白质水平显著下降[27]。这些研究结果表明, 在星形胶质细胞中, Aβ与CaSR共同作用促进了内源性Aβ的增加而CaSR的变构抑制剂会下调这种内源性增多。此外, 在人皮层非肿瘤神经细胞系-1A(human cortical nontumorigenic clonal strain-1A, HCN-1A)上取得了与在星形胶质细胞中相似的结果。研究发现, 无论纤维状的还是可溶性的Aβ都能显著地增加HCN-1A细胞胞体、轴突和树突中的内源性$A\beta_{1-42}$含量[27-29]。先用CaSR的变构抑制剂NPS 2143预处理, 再用纤维$A\beta_{25-35}$或可溶性$A\beta_{25-35}$处理HCN-1A神经元细胞, 结果发现, 无论胞体、轴突、树突还是高尔基体等细胞器中, 内源性$A\beta_{1-42}$都保持在正常生理水平, $A\beta_{1-42}/A\beta_{1-40}$值也处于正常范围。所以, 不管在人皮层神经细胞, 还是星形胶质细胞中, CaSR的变构抑制剂都能抑制Aβ/CaSR介导的$A\beta_{1-42}$内源性增加[24,29]。在该研究中, 通过对HCN-1A的细胞活性进行了测定, 发现NPS 2143还能抑制$A\beta_{25-35}$产生的细胞毒性, 保护HCN-1A神经细胞的活性和功能不受损害。

2 CaSR参与Aβ介导的炎症应激

一氧化氮(nitric monoxide, NO)是一种神经信号分子, 普遍存在于神经系统中[30]。星形胶质细胞在神经系统中占了一半以上的数量, 参与各种神经细胞之间的信号传递以及神经元与脑血管之间的能量和物质交换。在正常的生理情况下, 存在于星形胶质细胞中的NO通过上述的星形胶质–神经元–脑血管网络, 进行着Ca^{2+}信号的传递[24,31-32]。而在由外伤引起的脑损伤或者AD等慢性退行性神经疾病中发现, 星形胶质细胞能被炎症细胞因子如肿瘤坏死因子-α(tumo rnecresis factor-α, TNF-α)、干扰素-γ(interferon-γ, IFN-γ)和白介素-1β(interleukine-1β, IL-1β)等刺激, 并产生应答, 从而生成过量的NO[33-35]。一方面, NO能清除病理情况下产生的大量活性氧自由基(reactive oxygen species, ROS); 另一方面, 过剩的NO会通过星形胶质–神经元–脑血管网络, 损伤周边的神经元, 从而加重病情[25,31]。星形胶质细胞是



可溶性的Aβ与星形胶质细胞中的CaSR结合, 激活CaSR介导的MAPKs信号通路, 产生以下各种效应。(1)β切割酶的水平增加, Aβ产生级联放大。(2)HIF-1α•HIF-1β转录元件产生增加并出现细胞核内易位现象, 使VEGF-A表达增加, 从而影响脑血管的再生和血管通透性。(3)iNOS水平增加, $NO/ONOO^-$产生增多。相似的CaSR介导的Aβ增加还出现在神经元细胞上。可溶性Aβ刺激小胶质细胞释放TNF-α、IL-1β和IFN-γ等炎性因子, 作用于星形胶质细胞中的iNOS, 导致$NO/ONOO^-$的进一步累积。这些毒性因子以及脑血管的改变共同作用导致大量神经细胞的损伤和死亡。

Soluble Aβ binds to CaSR of astrocytes and activates CaSR-mediated MAPKs signaling pathways, which increase the expression level of beta-site APP-cleaving enzyme 1 (BACE1) and induce cascade amplification of Aβ. Both nuclear translocation of HIF-1α•HIF-1β transcriptional elements and expression of VEGF-A were increased. The increased VEGF-A will induce changes in regeneration of cerebrovascular and vascular permeability. The production of iNOS and $NO/ONOO^-$ were also elevated by MAPKs activation. Similar CaSR-mediated raising of Aβ was also found in neurons. On the other side, soluble Aβ stimulates microglias to release inflammatory factors such as TNF-α, IL-1β and IFN-γ, which stimulates iNOS, resulting in further accumulation of $NO/ONOO^-$. These toxic factors and changes of brain blood vessels caused damage and death of neural cells.

图1 CaSR参与Aβ对星形胶质–神经元–脑血管网络的损伤

Fig.1 CaSRs involved in the damage caused by Aβ in astrocyte-neuron-cerebral vascular network

NO在神经系统中的损伤机制的重要参与者。

AD中的特异性病理标志物$Aβ_{1-42}$能激活小胶质细胞, 介导TNF-α、IL-1β和IFN-γ等炎性因子产生, 这些炎性因子又作用于单体$Aβ_{1-42}$, 加速寡聚化[36]。同时, 聚合的可溶性$Aβ_{1-42}$可以作用于星形胶质细胞。目前, 在神经系统中主要有三种类型的NOS, 分别是诱导型一氧化氮合酶(inducible nitric oxide synthase, iNOS或NOS-2)、神经型一氧化氮合酶(neuronal nitric oxide synthase, nNOS或NOS-1)和内皮型一氧化氮合酶(endothelial nitric oxide synthase, eNOS或NOS-3)[30,36]。可溶性$Aβ_{1-42}$主要是使星形胶质细胞iNOS产生增加[37]。四氢生物喋呤(tetrahydrobiopterin, BH_4)是属于芳香族氨基酸羟化酶的辅酶, 是一氧化氮合酶的重要辅因子, 可以促进iNOS前体二聚化, 促使星形胶质细胞NO产生增加[30]。研究表明, 可溶性$Aβ_{1-42}$与星形胶质细胞中CaSR结合, 从而激活CaSR介导的MAPKs信号通路, 使BH_4水平上调, 导致NO释放增多, 并促使NO进一步转化成对各种神经细胞有强毒性的过氧亚硝酸盐阴离子(peroxynitrite anion, $ONOO^-$)。而CaSR的变构抑制剂NPS 89636能抑制BH_4的形成[37]。

随着$Aβ_{1-42}$淀粉样斑块的产生, 各种神经细胞产生了一系列的级联反应[38]。小胶质细胞开始释放炎性细胞因子和ROS; 星形胶质细胞CaSR介导的$Aβ_{1-42}$产生进一步增多[29]。增多的$Aβ_{1-42}$进一步诱导星形胶质细胞大量产生$NO/ONOO^-$。不仅如此, $Aβ_{1-42}$与神经营养因子受体p75(p75 neurotrophin receptor, $p75^{NTR}$)结合后介导的凋亡信号通路也被激活[32,36]。以上这些反应都使周围的神经元遭受了重创。

3 CaSR介导Aβ引起的血管内皮细胞生长因子(vascular endothelial growth factor, VEGF)过量产生

VEGF是一种脑源神经生长因子, 在生理情况下, 主要分布于神经元和脉络丛, 与血管的生成有着密切关系。在缺血再灌注损伤中, VEGF能降低缺血造成的高氧自由基, 从而保护神经细胞不受损伤。在肌萎缩性脊髓侧索硬化症中, VEGF还能保护运动型神经元免受谷氨酸盐的损害[39]。在AD发病进程中, 记忆的编码和检索功能(memory encoding and retrieval functions)会随着病情的加重逐渐丧失, 而这些认知功能障碍的形成都已被证明与VEGF的缺失有关[40]。VEGF除了是一种神经生长因子, 与脑内神经元和胶质细胞等细胞的生长和迁移有关之外, 还与诱导血管增生有极大关系, 其中又以VEGF-A的诱导作用为最强, 而血管增生已经被证明是AD等神经退行性疾病的发病机制之一[41-42]。同时, 过剩的VEGF也被发现作为毒性因子参与着众多脑部疾病的发生。局部缺血和神经炎症等造成的脑损伤中, VEGF过多能加速神经元的损伤以及血脑屏障功能的丧失[42-44]。

对AD发病机制的研究发现, 在Aβ积聚的AD脑组织中也确实存在VEGF-A水平的明显上调, 而且在易患AD的APOE4(apolipoprotein E4)基因型个体的血浆中也发现了VEGF水平上调的现象[44]。小胶质细胞、星形胶质细胞和神经元中的VEGF-A水平增高和分泌增加也被作为AD的一项病理现象。研究发现, 可溶性$A\beta_{1-42}$和可溶性$A\beta_{25-35}$都能有效刺激星形胶质细胞, 使其过量产生和释放VEGF-A[45]。VEGF-A能特异性地促进血管细胞分裂、增殖和迁移, 从而增加血管密度, 促进血氧的传递和交换。而过多的VEGF-A则会损害血脑屏障, 使其功能丧失[45]。

用CaSR的拮抗剂NPS 2143和激活剂NPS R-568分别处理星形胶质细胞, 对其下游机制进行研究, 结果发现, CaSR拮抗剂NPS 2143能完全抑制$A\beta_{25-35}$和$A\beta_{1-42}$导致的VEGF-A过量释放, CaSR激活剂NPS R-568的作用与NPS 2143完全相反[45]。VEGF的产生可由两种通路进行调节。在含氧量正常的培养条件下, *VEGF*基因可由经p42/p44 MAPKs通路磷酸化的转录因子特异性蛋白1/转录因子激活蛋白-2(transcription factorspecific protein 1/transcription factor activator protein, Sp1/AP-2)在其近启动子区域进行激活。然而在低氧条件下, MAPKs能磷酸化修饰缺氧诱导因子-1(hypoxia-inducible factor-1, HIF-1), 进而低氧诱导产生HIF-1α•HIF-1β异质二聚体形式的转录因子[45]。在可溶性$A\beta_{25-35}$处理后的星形胶质细胞中发现了明显的HIF-1α•HIF-1β转录元件的细胞核内易位现象, 而NPS 2143能抑制这种易位现象[45]。以上证据表明, Aβ作为一种特异性的配体通过与CaSR特异性地结合, 从而激活MAPKs通路来增加HIF-1的表达, 促进VEGF含量增加和过量释放。过剩的VEGF-A改变了血脑屏障的通透率, 使脑组织内的缺血情况更加恶化, 加速神经元的死亡, 从而加重了AD的发病进程[39,46]。

4 总结与展望

以上研究结果表明, CaSR与可溶性Aβ共同在AD的发病过程中起着重要的作用。可溶性$A\beta_{1-42}$寡聚体诱导激活星形胶质细胞上的CaSR介导的信号通路, 一方面导致一系列神经毒性物质的过量产生和释放, 如可溶性Aβ寡聚体和$NO/ONOO^-$等, 而这些神经毒性因子又作用于神经元; 另一方面产生VEGF-A, 导致血管增生, 改变血脑屏障。CaSR参与Aβ对星形胶质–神经元–脑血管网络的改变(图1), 从而在AD发病进程中的多个阶段中起着重要的作用[47]。

虽然CaSR在AD发病机制中的作用已取得了一定的进展, 但目前的研究主要集中在细胞水平, 大部分是在细胞系里得到的结果, 缺乏组织和在体水平的研究。除了文章中提到的三方面外, CaSR是否参与Aβ介导的其他病理过程以及CaSR与其他AD致病分子之间的作用都值得进一步的研究。例如, CaSR是否参与AD中另一个重要的致病因子Tau蛋白质的磷酸化以及神经纤维样缠结的形成? 在Aβ存在下, CaSR如何介导突触的反应以及突触可塑性损伤? CaSR的下游信号通路比较复杂, CaSR被认为是在正常和病理条件下调节细胞命运的受体之一, 在不同的组织或受到不同内源性变构剂的作用后, CaSR可能选择性的激活一到两条信号通路, 引起细胞的增殖或死亡[48]。在AD发病过程中, CaSR表达量是如何变化的? 它又受到那些因素的调节? 除了文中提到的MAPKs信号通路, 其他信号通路是如何被调节的? 这些都值得进一步研究。

CaSR的变构抑制剂作为一种药物, 能通过血脑屏障。目前的研究结果表明, 具有神经毒性的$A\beta_{1-42}$的聚合和扩散能被CaSR的变构抑制剂有效地阻断。

表3 CaSR拮抗剂阻断AD病理损伤

Table 3 The prevent role of CaSR antagonist in the impairments of AD

细胞模型 Cell models	拮抗剂 Antagonists	结果 Results	参考文献 References
Human cortical nontumorigenic clonal strain 1A	NPS 2143	Inhibit endogenous production of $A\beta_{1-42}$ induced by $A\beta_{25-35}$; protect neurons from cytotoxicity of Aβ.	[24]
Normal adult human astrocytes	NPS 2143	Inhibit endogenous production of Aβ.	[27,29]
	NPS 89636	Inhibit formation of BH4 and iNOS, and decrease inflammatory stress response induced by $NO/ONOO^-$.	[37]
	NPS 2143	Inhibit of Aβ induced excessive release of VEGF-A and nuclear translocation.	[25,45]

$NO/ONOO^-$和VEGF-A引起的神经细胞损伤也能被CaSR的变构抑制剂有效逆转(表3)。所以, 如NPS 2143等CaSR变构抑制剂有望成为治疗散发性AD的有效药物, 但是CaSR变构拮抗性药物的副作用也不应被忽视。CaSR变构拮抗性药物会过度激活甲状旁腺素的释放, 促进骨生成和溶解, 诱导骨质疏松的形成, 同时造成轻微的甲状旁腺功能亢进[49]。所以, 针对上述副作用, CaSR抑制类药物有待改良, 而CaSR介导的信号通路上特定位点的靶点药物也有待开发, 这可能会成为治愈AD的关键。

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