Review

Evaluation of rage isoforms, ligands, and signaling in the brain

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Abstract

Since the identification of the receptor for advanced glycosylation end products (RAGE) in 1992, there have been tremendous strides made in our understanding of the role RAGE receptors play in a variety of physiological and pathological processes. Despite such progress, several fundamental aspects of RAGE expression and RAGE function remain largely unanswered. In particular, while multiple forms of the RAGE receptor are known to exist, little is known with regards to how these different isoforms of the RAGE receptor work together to mediate RAGE signaling. For example, some forms of the RAGE receptor may promote deleterious feed-forward pathways, while others may serve to inhibit deleterious activation of the RAGE receptor. Additionally, important questions remain with regards to the intracellular domain of the full-length RAGE receptor, and the specifics surrounding how intracellular signaling pathways become activated via the RAGE family of receptors. The focus of this review is to address each of these important issues, as well as other key aspects of RAGE biology, and discuss how they are important for both our understanding of the physiological and pathological roles of RAGE signaling within the brain.

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1. Introduction

In attempting to identify the presence of receptors for glycated proteins in the bovine lung, investigators described the presence of a high affinity receptor present on the cell surface. This receptor was subsequently purified and defined as the full-length receptor for advanced glycosylation end products (RAGE). Upon sequencing, the RAGE receptor was characterized as being a member of the immunoglobulin superfamily [1–4], and was observed to display a high degree of structural and sequence homology to the neural adhesion molecules (N-CAM). Structurally, each of the principle isoforms of the RAGE receptor contains an N-terminal V-type domain followed by 2 C-type domains (Fig. 1). The V-type domain is believed to be the principle site for ligand binding, although the C-type domains likely play a key role in stabilizing the V-domain for ligand interactions. RAGE is a multiligand receptor, which binds a diverse range of molecules, with a preference for ligands that are prone to aggregation and post-translational modifications (oxidation, glycosylation, etc.). The full-length form RAGE has a single transmembrane domain that contains a 43 amino acid intracellular domain (Fig. 1). This short cytoplasmic tail of the full-length RAGE receptor has no known phosphorylation sites, G-protein binding sites, or kinase binding sites. Regardless, the cytoplasmic tail is absolutely essential for the intracellular signaling.

2. RAGE isoforms

When discussing RAGE, it is important to keep in mind that there are at least 3 major forms of RAGE. These different RAGE isoforms are generated as the result of alternative splicing and can loosely be defined as being the full-length RAGE, dominant negative RAGE (DNRAGE), and secretory RAGE (sRAGE) (Fig. 1). Each of these forms of RAGE contain the V-type and C-type domains, and are presumably able to bind RAGE ligands with similar affinity (Fig. 1), although this remains to be fully elucidated experimentally. Each of these forms of the RAGE isoforms is generated as the result of splicing from a single RAGE gene, although some differential forms of RAGE in mice may result as the result of protein cleavage instead of splicing [5]. It is important to keep...
in mind that the splicing independent generation of RAGE isoforms in mice remains controversial, with recent studies from our laboratory confirming the possibility of RAGE splicing in the mouse brain (J.N. Keller, unpublished observations). It is interesting to note that splicing variants for each of the 3 well established RAGE isoforms are also known to exist [6–11]. In particular, studies from our laboratory have identified the presence of at least 3 novel forms of RAGE in the human brain [9]. These specialized forms of RAGE may be particularly important to a variety of pathological conditions [6–11], and are discussed in more detail below. Additionally, polymorphisms in RAGE are also known to exist, with polymorphisms profoundly affecting the function of RAGE [12–14]. In order to develop an accurate understanding of RAGE signaling in the brain and central nervous system (CNS), it is important to understand the interaction, expression, and function of each of these RAGE isoforms.

2.1. Full-length RAGE

Full-length RAGE is by far the most studied of the 3 major forms of RAGE, with numerous studies identifying a role for full-length RAGE as a direct mediator of physiological as well as pathological responses [1–4]. The full-length form of RAGE contains a single transmembrane region, followed by a short intracellular domain (Fig. 1). The intracellular domain of RAGE is necessary for many forms of RAGE signaling, including the activation of nuclear factor-κB (NF-κB), mitogen activated protein kinase (MAPK), and Jun-N-terminal kinase (JUNK) [15–20]. Interestingly, the activation of these different pathways by full-length RAGE may involve members of the Rho-family of GTPases as well as RAS [1–4,15–20]. At the present time, it is assumed that the intracellular domain of the full-length RAGE receptor serves as a scaffolding for the initiation of signal transduction. For example, a recent report suggests that a D-domain on the cytoplasmic tail of RAGE may provide for direct docking of MAP kinase to the cytoplasmic domain of full-length RAGE [21]. However, it is important to point out that these studies were conducted using robust expression of only the intracellular domain of RAGE for binding analysis. As such, these data may not accurately reflect what occurs with the actual full-length RAGE receptor. A number of RAGE ligands, as well as RAGE activation itself, can induce oxidative stress. Because reactive oxygen species (ROS) are sufficient to stimulate signal transduction, it is critical to elucidate how ROS might be involved in RAGE signaling. For example, it will be important to determine if there are ROS-dependent as well as ROS-independent modes of RAGE signaling. Additionally, studies need to elucidate the role of the intracellular domain in these potentially different modes of signal transduction. While studies with antioxidants have demonstrated a necessity for ROS in some forms of RAGE-induced signal transduction [22,23], they have not provided an overview as to how antioxidants affect the other established RAGE signaling pathways. Indeed, most studies have focused on the ability of antioxidants to prevent or ameliorate the activation of NF-κB in response to RAGE activation, with little known about the role of ROS in other downstream signaling pathways. Additionally, it is unclear whether ROS are a general mediator of RAGE signaling, or whether certain RAGE ligands may have a higher dependence upon ROS generation for the initiation of downstream pathways. As mentioned previously, at present, the proteins which

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**Fig. 1.** There are multiple isoforms of the RAGE receptor. The 3 dominant isoforms of the RAGE receptor are known as full-length RAGE, soluble RAGE (sRAGE), and dominant negative RAGE (dnRAGE). Each of these receptors contains a V-type domain and 2-C type domains for ligand binding, but each RAGE isoform differs considerably in cellular function. The sRAGE receptor is released from the cell and allowed to interact with RAGE ligands prior to their interacting at the plasma membrane. The DNRAE lacks the intracellular signaling domain, and therefore binds RAGE ligands without directly transducing a signal. Together, the presence of these 3 main isoforms of RAGE suggests that the ability of any RAGE ligand to induce RAGE signaling depends on the coordinated effects of the different RAGE receptors. At least 3 additional forms of RAGE are found in the human brain (RAGEb, sRAGEb, DNRAEb), arrows indicate the areas of each brain isoform that is unique.
bind to the intracellular domain of RAGE, under stimulated as well as unstimulated conditions, have not been identified. Identifying such proteins is absolutely paramount to understanding RAGE induced signal transduction for both physiological as well as pathological conditions. It will also be important to identify the role of ROS as a potential regulator of these different proteins which may bind to the intracellular domain of full-length RAGE.

Signaling through full-length RAGE is known to be vital for a number of physiological as well as pathological processes. These include chemotaxis, angiogenesis, inflammation, neurite outgrowth, apoptosis, and proliferation [1–4]. It is extremely interesting that the same receptor–ligand interaction can have such varied and cell type-specific effects. Presumably the ability of RAGE activation to induce these varied responses is due at least in part to the fact that a given signal transduction pathway can have extremely cell type specific effects. For example, it is well established that the activation of NF-κB is absolutely essential for viability in some cells, and induces apoptotic pathways in other cells [24]. In most paradigms, it has been determined that one of the most rapid effects of RAGE activation is the generation of more full-length RAGE on the cell surface. The presence of more full-length RAGE presumably results in greater and more sustained signaling through the full-length RAGE receptor. In this scenario, once full-length RAGE becomes activated, its signaling is rapidly accelerated and potentially difficult to ameliorate. Because sRAGE and DNRAGE are both able to suppress signaling through the full-length RAGE receptor, it is likely that one of their main functions is to serve as vital inhibitors of full-length RAGE activation.

2.2. Soluble RAGE

The soluble form of RAGE contains the same V-type and C-type regions found on the other isoforms of RAGE (full-length RAGE, DNRAGE), but lacks the transmembrane domain (Fig. 1). As a result, sRAGE is released into the extracellular space and allowed to interact with RAGE ligands prior to their potential binding to full-length RAGE (Fig. 2). As a result, sRAGE is presumably able to suppress the activation and signaling of full-length RAGE, by sequestering the RAGE ligands prior to their interaction with the full-length RAGE receptor. An alternative mechanism by which sRAGE can modulate signaling full-length RAGE is by altering the generation and maturation of potential RAGE ligands. This is perhaps best illustrated in the analysis of sRAGE and beta amyloid (Aβ). It is well established that Aβ (a small peptide of 40–42 amino acids) undergoes increasing degrees of aggregation, which is associated with fibrillation and oligomerization [25,26]. As the aggregation of Aβ increases, it begins to form higher ordered structures, ultimately generating highly cross-linked and insoluble plaques (Fig. 2). The different forms of Aβ (soluble/insoluble) are known ligands for full-length RAGE (Fig. 2), with signaling through RAGE believed to play a potentially important role in the Aβ pathogenesis observed in Alzheimer’s disease (AD) [27–33]. It is important to keep in mind that in this model full-length RAGE is believed
to bind highly crosslinked and aggregated Aβ (Fig. 2), and may not be stimulated to the same degree in response to monomeric and soluble Aβ (Fig. 2). Recent studies indicate the soluble forms of RAGE are able to not only bind Aβ in the earliest stages of aggregation, but also demonstrate that the binding of soluble RAGE to Aβ dramatically ameliorates Aβ aggregation and prevents the generation of insoluble Aβ structures (Fig. 2) [34]. As a result, soluble RAGE is able to suppress full-length RAGE activation by sequestering RAGE ligands before they encounter the membrane form of RAGE, and also by decreasing the generation of RAGE ligands (Fig. 2). In future studies, it will be important to elucidate the ability of sRAGE to prevent other potential RAGE ligands from initially forming.

Additional evidence in a variety of transgenic mouse models suggest that sRAGE has important inhibitory effects on full-length RAGE signaling. For example, administration of soluble RAGE into the circulatory system has been demonstrated to suppress atherosclerosis, atherogenesis, increase wound healing, and increase peripheral nerve regeneration [1-4,35-37]. Presumably, in each of these models, sRAGE serves to ameliorate the activation of full-length RAGE by preventing the interaction of RAGE ligands with the full-length form of the receptor. Additionally, we propose that the beneficial effects of sRAGE may be manifest by ameliorating the generation of RAGE ligands (similar to what occurs with Aβ). For example, the oxidation of low density lipoprotein molecules or the generation of AGE-modified proteins increases their propensity to aggregate or form higher order complexes. The presence of sRAGE may play a role in ameliorating such aggregation and thereby prevent the formation of potentially more efficacious activators of RAGE signaling. Consistent with such a scenario, infusion of sRAGE into the circulatory system decreases the binding of AGE modified proteins to the endothelial cell surface [38]. Indeed, at the time of this publication, a host of new reports describing alterations in the levels of circulating sRAGE correlating with the onset or severity of pathogenesis have strengthened the importance of sRAGE to a wide variety of disorders [39,40].

A newly published report suggests that the greatest expression of sRAGE in the brain is localized to neuronal cytoplasm [41]. Studies from our laboratory have demonstrated that at least 2 forms of sRAGE (nearly identical) are expressed in the human brain, with sRAGE mRNA observed to be at least 3 fold higher than other forms of RAGE [9]. Such findings suggest that healthy neurons may retain a healthy reserve of sRAGE to release upon encountering potentially stressful stimuli such as AGEs or Aβ. Together, these data highlight the importance of RAGE activation to a number of pathogenic processes, and highlight the potential therapeutic role for sRAGE in these different pathogenic conditions. Recent studies have demonstrated the ability of sRAGE administration to the circulatory system of rodents is able to prevent beta amyloid from the circulatory system from crossing into the brain [42] and ameliorate the formation of diffuse plaques [43]. Despite such reports, one can make the case that relatively little is known experimentally about the potential for sRAGE as an ameliorator of Aβ pathogenesis in vivo. For example, does sRAGE suppress the formation and toxicity of soluble and insoluble oligomeric forms of Aβ? Is sRAGE able prevent the formation of neuritic plaques, or is it only capable of suppressing diffuse plaque formation? Similarly, it is unclear whether the ability of sRAGE to suppress plaque formation requires that it be present in the circulatory system or whether sRAGE mediates its effects directly in the brain?

2.3. Dominant negative RAGE

The least characterized and understood of the RAGE isoforms is the DNRAE. This form of RAGE is identical to full-length RAGE, only lacking the short intracellular domain of full-length RAGE (Fig. 1). Presumably, this form of RAGE competes for the binding of RAGE ligands and prevents the activation of full-length RAGE due to its lack of an intracellular domain (Fig. 2). The majority of our understanding of this form of the RAGE receptor has come from transfection studies, which demonstrated that dramatic upregulation of DNRAE decreases full-length RAGE activation [27,29]. However, it is important to note that these studies were short-term studies (less than 48 h) and may not provide an accurate representation of the in vivo paradigm. For example, a number RAGE ligands are deleterious to most cell types (AGEs, oxidized lipoproteins, Aβ, etc.), and binding of these ligands to DNRAE would be expected to hold them at the cell surface. While holding of such ligands at the cell surface and preventing their initial binding to full-length RAGE may be beneficial during the initial periods of ligand binding, the long-term effects may be less advantageous. For example, one could imaging that the localization of these ligands at the cell surface may allow for the initiation of further ligand recruitment, oxidation, and aggregation. This may be particularly true for Aβ and oxidized lipoproteins, which then promote the activation of full-length RAGE. However, experimentation is needed to clarify the validity of this hypothesis. In a recent report, we have demonstrated that the level of DNRAE expression in the human brain is similar to full-length RAGE [9]. However, little is currently known about how the ratio of full-length RAGE and DNRAE is altered in response to physiological and pathological stimuli. Such data may be important in identifying a role for DNRAE as a suppressor of full-length RAGE signaling.

2.4. Additional splice variants of RAGE

While there are 3 predominant forms of RAGE, a growing number of studies have highlighted the presence of additional RAGE splice variants [6-11]. For example, alternatively spliced forms of sRAGE and full-length RAGE have been reported [6-11]. Interestingly, three of these studies demonstrated evidence of alternative RAGE splicing in brain astrocytes [7], neural cells [8], and human brain tissue [9]. These alternative splicing events result in the truncation of exons as well as the failure to remove intronic sequences from RAGE mRNA. It is highly likely that the protein products
2.5. Polymorphisms in RAGE

Another rapidly growing area of RAGE research indicates a role for polymorphisms in RAGE as mediators of altered RAGE function. Additional reports have linked RAGE polymorphisms with multiple pathological conditions. For example, a number of studies have now identified the presence (and potential linkage) of a G82S polymorphism in RAGE to both rheumatoid arthritis and diabetes [12–14]. The G82S polymorphism is located within the V-type immunoglobulin domain of RAGE and appears to slightly increase the binding of RAGE ligands to the surface of Chinese hamster ovary cells, and dramatically elevate the activation of inflammatory signaling pathways [14]. It has therefore been speculated that the presence of RAGE polymorphisms may shift RAGE signaling to being more pathological in nature, with elevated inflammation and oxidative stress from RAGE activation contributing to the induction of diabetes and rheumatoid arthritis. It is unclear whether polymorphisms in RAGE potentially contribute to alterations in brain homeostasis, although it is enticing to speculate on the presence of RAGE polymorphisms contributing to alterations in oxidative stress and inflammatory signaling in the brain, and potentially contributing to the development of age-related disorders of the CNS.

3. RAGE ligands

A large number of ligands for RAGE have been identified in the brain as well as other tissues. A number of studies have demonstrated that advanced glycosylation end product (AGE), S100/calgranulins, Aβ, and amphoterin are all natural ligands for RAGE [1–4,44]. Although numerous studies have analyzed the ability of these different ligands to stimulate RAGE, currently very little is known about the contribution these different ligands play in mediating RAGE signaling within the CNS. Additionally, little is known about the potential synergistic or inhibitory effects of the different RAGE ligands on the RAGE-mediated signaling. Understanding more of the specifics that currently surround the effects of these different ligands, and elucidating their potential interactions with one another, is clearly much needed to accurately understand RAGE signaling in the brain.

3.1. Advanced glycosylation end products (AGEs)

AGEs are formed from a series of glycosylation and oxidation reactions with sugars and amino acids, with the amino acid side chains of lysine, histidine, and arginine particularly vulnerable to modification by AGEs [1–4,45–47]. The most common sugars for AGE formation include glucose, fructose, and triose-phosphates. Each of these sugars form a Schiff base adduct that is modified to a ketoamine Amadori product. This reaction is referred to as non-enzymatic glycosylation or the Maillard reaction, and generates reversible glycosylation. Once exposed to oxidation reactions, these glycosylation events form fluorescent products that are then defined as being AGEs. These AGEs form irreversible modifications to proteins, which render proteins resistant to protease digestion and elevate protein crosslinking. Interestingly, AGEs have been demonstrated to accumulate in the intracellular as well as extracellular space as a part of normal brain aging [47]. Age-related disorders such as AD demonstrate an exacerbation of AGEs as compared to age-matched controls, with AGEs associated with numerous pathological features of the AD brain. Interestingly, there is a strong association between Aβ deposition and AGEs in the brain [45]. This may be particularly important given the fact that Aβ and AGEs are both ligands for RAGE, and may therefore promote a synergistic activation of RAGE. Evidence for potential synergy is highlighted by the fact that AGEs are capable of accelerating the nucleation-dependent polymerization of Aβ [45].

Interactions between AGEs and the various RAGE isoforms have been well documented outside of the CNS. For example, studies have demonstrated that AGEs interact with full-length RAGE and sRAGE in tissues of the vasculature [1–4,45–47]. Additionally, studies have demonstrated that many effects of AGE administration require the presence of the cytoplasmic tail of full-length RAGE [1–4,45,48]. Together, these studies suggest that sRAGE and DNRAGE likely play key roles in inhibiting signaling mediated by the interaction of AGEs with full-length RAGE [1–4,45,48]. Because such signaling can induce a diverse array of physiological responses, ranging from proliferation to apoptosis, it is likely that AGE-RAGE interactions are important to brain aging and age-related diseases of the CNS. However, at the present time little is known about the role and effects of AGEs in physiological or pathological responses observed in the brain.

3.2. S100/calgranulins

The S100/calgranulin proteins are closely related, and have been best characterized for their role in intracellular calcium binding [1–4,49–52]. However, in addition to binding calcium these proteins also have an established role in protein export [50,51]. Once outside of the cell, members of the S100/calgranulin family of proteins can potently bind to the different RAGE isoforms. Members of the S100/calgranulin protein are highly expressed throughout the brain and CNS, with expression localized predominantly to astrocytes and specific neuron populations [49–52]. The binding of S100/calgranulins
to RAGE has been demonstrated to potently stimulate neurite outgrowth and neuron survival in a variety of in vitro studies [49–52]. Additionally, S100/calgranulin proteins have been demonstrated to have multiple effects on microglia including the stimulation of nitric oxide release as well as the activation of multiple signal transduction pathways [53,54]. Interestingly, it appears that in some models the activation of NFκB, which is RAGE dependent [53,54], can be separated from other S100-induced events such as nitric oxide release. The concentration of S100/calgranulin within the extracellular space appears to be a particularly important factor in predicting its overall effect on cellular homeostasis, with low concentrations driving more beneficial effects (neurite outgrowth), and high concentrations inducing more deleterious events (inflammatory signaling). In future studies, it will be important to elucidate the relative contribution of differential RAGE activation to this dose-dependent variability in S100/calgranulin mediated signaling. For example, it will be important to elucidate whether low concentrations of S100/calgranulin may selectively bind to a specific RAGE variant.

3.3. Amphoterin

Amphoterins are members of the high mobility group 1 (HMG-1) nonhistone chromosomal DNA binding proteins [52,55], which have been demonstrated to potently induce inflammatory signaling in the CNS as well as non-CNS tissues [52,55–57]. In general, the term amphoterin is generally reserved for the membrane bound forms of HMG-1 protein, although this term is used loosely in the literature. The members of the amphoterin/HMG-1 group of proteins are expressed at their highest levels in the CNS during embryonic development [52,55–57], although they may be significantly elevated within the CNS in response to injury [52,55–57]. The ability of amphoterin/HMG-1 proteins to induce inflammatory responses can be RAGE dependent, as well as RAGE independent, contributing to the complexity surrounding the effects of this RAGE ligand. Similar to S100/calgranulin proteins, amphoterin is also a potent inducer of neurite outgrowth [49,55–57]. It is important to note that there is a tremendous overlap between neuronal RAGE expression and the neuronal expression of amphoterin, during the development of the CNS. Taken in conjunction with the ability of amphoterin/HMG-1 proteins to induce neurite outgrowth, numerous investigators have suggested an important physiological role for amphoterin/HMG-1 signaling through RAGE during neuronal development. It appears that amphoterin/HMG-1 signaling through RAGE can therefore have both beneficial as well as potentially deleterious effects upon cellular homeostasis. Additionally, it appears that the ability of the same stimuli (amphoterin/HMG-1) to induce divergent responses is due to the cell type specific nature of RAGE signaling. For example, in neurons, the binding of amphoterin complexes results in beneficial neurite outgrowth, while in microglia that same ligand induces potentially deleterious increases in inflammatory signaling. Such data raise the possibility that expression of the different RAGE isoforms may be altered at the cell surface in a cell type specific manner, and account for the dramatic differences in outcome following exposure to the same RAGE ligand.

3.4. Beta amyloid

Aβ is believed to play an important role in promoting increased oxidative stress and mediating the increased neuron death observed in the AD brain [25–27]. Aβ is a peptide fragment, principally 40 or 42 amino acids in length that is generated as the direct result of proteolytic cleavage from the β-amyloid precursor protein (APP) [25,26]. Increased levels of Aβ are observed in AD, and are increased by a gradual and progressive increase in the steady-state levels of Aβ as the result of altered Aβ synthesis and decreased catabolism of Aβ [25,26]. As mentioned previously, once generated, Aβ is capable of generating complexes with increasingly complicated structure (Fig. 3).

A number of studies have identified that RAGE receptors bind beta amyloid, binding monomeric, fibrillar and presumably oligomerized Aβ (Fig. 2). RAGE immunoreactivity has also been demonstrated at the sites of Aβ deposition [27–32]. Additionally, RAGE has been suggested to be a principle receptor for binding Aβ at the cell surface [28–30]. The binding of Aβ to the cell surface has also been suggested to increase Aβ aggregation at the plasma membrane. Together, these studies have suggested that Aβ signaling through RAGE is responsible for a significant amount of Aβ induced oxidative stress, inflammation and neurotoxicity [27–32]. However, it is important to point out that these previous studies have excluded the potential role of sRAGE and DNRAGE to these previous findings. For example, it is unclear whether the RAGE immunoreactivity at the site of Aβ deposition is full-length membrane bound RAGE, DNRAGE, or represents the binding of sRAGE. The binding of sRAGE to these Aβ deposits could indicate an attempt to inhibit further Aβ deposition, or serve as a means of preventing the binding of Aβ to the full-length RAGE receptor. A recent study has demonstrated the ability of sRAGE to not only bind Aβ, but also prevent its aggregation and its ability to form higher order complexes [34]. As such, the ability of sRAGE to bind Aβ may dramatically interfere with the ability of Aβ to bind to membrane bound RAGE and directly transduce potentially toxic signal transduction. Additionally, the potential role of endogenous DNRAGE in decreasing Aβ signal transduction is poorly understood. It is likely that the expression of sRAGE and DNRAGE dramatically increases in response to elevating levels of Aβ, and suggest that increased levels of sRAGE or DNRAGE could potentially serve as a potential therapeutic target for successfully suppressing Aβ toxicity. It will be important to elucidate the contribution of alterations in the ratio of sRAGE/DNRAGE/RAGE and Aβ as a potential trigger for the initiation of Aβ toxicity in AD.

In AD, it has been reported that there is an increased level of circulating IgGs which bind RAGE peptides [58]. Interestingly, there appeared to be a cross reactivity with IgGs which recognize Aβ peptides and RAGE peptides, suggesting that the
antibodies may be being made in response to a common protein complex. It is important to point out that the increase in circulating RAGE IgGs were demonstrated to not occur as the result of a linkage between diabetes and AD [58]. When mice were immunized with an AGE-modified protein, the mice generated IgGs against the RAGE receptor and Aβ [59]. These studies provide an additional linkage between circulating levels of anti-RAGE and anti-Aβ IgGs. Such linkages have caused some investigators to speculate that anti-RAGE IgGs may have both diagnostic as well as therapeutic potential in AD. It will be important in future studies to address the mechanisms responsible for the linkage between the levels of anti-RAGE and anti-Aβ IgGs in AD subjects. Additionally, studies are needed to elucidate the potential function, or effects of circulating RAGE, with the function of RAGE within the brain.

4. Coordinated signaling through RAGE

Based on the large number of RAGE isoforms, the presence of additional splice variants for each of these RAGE isoforms, and the presence of RAGE polymorphisms, it is clear that when one speaks of RAGE signaling one is referring to an outcome that is the result of the interplay between all of these different factors. In the first set of factors, one has to consider the relative amounts of the different RAGE ligands which are present within a specific area of the brain (Fig. 3). In conducting such analysis it is important to not only measure the amount of the individual RAGE ligands which are present, but also begin to elucidate the very real possibility that these ligands may have synergistic or inhibitory effects towards one another (Fig. 3). The effects of these different ligands on RAGE signaling is regulated, at least in part, by the ability of sRAGE to bind the ligand prior to reaching the full-length RAGE receptor (Fig. 3). By increasing or decreasing the ability of sRAGE to interact with these different RAGE ligands, it is likely that sRAGE serves as a direct and profound modulator in determining the amount of ligand which encounters RAGE receptors on the cell surface. Once the RAGE ligands encounter the cell surface of CNS cells, there will likely be some shared responses, but the ultimate effect on cellular homeostasis is likely to be cell type specific (Fig. 3). For example, Aβ signaling through RAGE in a microglia is likely to increase oxidative burst and proliferation, while the same ligand–receptor interaction in a neuron is likely to promote apoptosis (Fig. 3). Evidence also suggests that under conditions of high level RAGE expression at the cell surface, RAGE activation may induce growth arrest and even apoptosis in microglia [43,49,60,61]. Accurately understanding and targeting of RAGE signaling in potential therapies will clearly rely on the utilization of models which take into account each of these complexities that currently cloud our understanding of RAGE in the CNS.

Because the vast majority of previous studies have discussed RAGE in terms of being one receptor, considerable confusion has emerged in this growing field. It is important to note that some potentially important assumptions have also been made in previous studies. For example, the vast majority of immunohistochemical studies have used antibodies which are not capable of distinguishing between the different RAGE isoforms. As a result, it is unclear if immunostaining represents the expression of sRAGE, DNRAGE or full-length RAGE. As outlined above, such identification and characterization is
critical to accurately interpreting experimental results, as each of these forms of RAGE can have dramatically different effects on cellular function.

It is vital to understand the overall balance of RAGE expression in physiological and pathological conditions, as it is likely that subtle shifts in the ratio of full-length RAGE and the amount of inhibitory forms of RAGE, significantly impacts intracellular signaling. Furthermore, the potential loss of inhibitory forms of RAGE (sRAGE, DNRAGE) in the CNS has never seriously been addressed in previous immunohistochemical studies, thus findings from these previous studies may have inadvertently added confusion to our understanding of how RAGE expression is altered in both the development of the CNS, as well as the inflammation and neurodegeneration evident in the various CNS disorders such as AD.

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