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Differential effects of "Advanced glycation endproducts" and β-amyloid peptide on glucose utilization and ATP levels in the neuronal cell line SH-SY5Y

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Summary. β-amyloid peptide (Aβ) and "Advanced glycation endproducts" (AGEs) are components of the senile plaques in Alzheimer's disease patients. It has been proposed that both AGEs and Aβ exert many of their effects, which include the upregulation of pro-inflammatory cytokines, through RAGE ("receptor for advanced glycation endproducts"). To investigate whether Aβ and AGEs cause similar or identical effects on cell survival and energy metabolism, we have compared the effects of a model-AGE and Aβ on cell viability, ATP level, glucose consumption and lactate production in the neuroblastoma cell line SH-SY5Y. The results show that AGEs and Aβ increase glucose consumption and decrease ATP levels in a dose dependent manner. Furthermore, both compounds decrease mitochondrial activity measured by the MTT assay. However, only AGEs decrease the number of cells and significantly increase lactate production. These data indicate that both AGEs and Aβ can cause differential disturbances in neuronal metabolism, which may contribute to the pathophysiological findings in Alzheimer's disease. However, their signalling pathways are apparently quite distinct, a fact which should stimulate a more detailed investigation in this field, e.g. for the purpose of a rational design of potential "neuroprotective" RAGE antagonists.

Keywords: Glycation, amyloid, glucose, lactate, ATP.

Abbreviations

AD Alzheimer's disease, *AGE(s)* advanced glycation endproduct(s), *APP* amyloid precursor protein, *BSA* bovine serum albumin, *CML* carboxymethyllysine, *CSF* cerebrospinal fluid, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide.

Introduction

The amino groups of proteins, particularly on lysine residues, react non-enzymatically with reducing sugars. This posttranslational modification, termed ''non-enzymatic glycosylation'', ''glycation'' or ''Maillard reaction'' leads, via reversible Schiff-base adducts, to the formation of protein-bound Amadori products. Through subsequent oxidations and dehydrations, a heterogeneous mixture of fluorescent and brown products containing nitrogen- and oxygen-containing heterocycles is formed, the so-called ''Advanced Glycation Endproducts'' (AGEs). These latter reactions are irreversible and cause protease-resistant cross-linking of peptides and proteins, leading to protein deposition and amyloidosis (Brownlee, 1995). Deposition of AGE-crosslinked insoluble protein aggregates including β_2-microglobulin is characteristic of dialysis-related amyloidosis or islet amyloid polypeptide in Type II diabetes mellitus (Miyata et al., 1994a). In Alzheimer's disease (AD), AGEs accumulate on β-amyloid plaques in the brain, and in microglia and astrocytes in their vicinity (Dickson et al., 1996). Although the degree of AGE modification of proteins in senile plaques has not been quantified, it is likely to be much higher than in plasma proteins, since the half-life of proteins in the senile plaques is estimated to be approximately 30 years compared to the much shorter halflife of 1–2 months for most of the plasma proteins. The proteins of a senile plaque are available for AGEs modification as are other long-lived proteins such as eye lens crystallin and in β_2-microglobulin deposits in dialysis patients (Miyata et al., 1994b; Saxena et al., 2000).

It has been proposed that AGEs transmit their signals through the ''receptor for advanced glycation endproducts (RAGE)'' (Schmidt et al., 1994) which is a multi-ligand member of the immunoglobulin superfamily of cell surface receptors (Schmidt et al., 2000). Its repertoire of ligands include AGEs, amphoterins, S100/calgranulins and fibrillar peptides including Aβ from the AD brain (Yan et al., 1998). Aβ, derived by proteolytic cleavage of the amyloid precursor protein (APP), is the major protein component of senile plaques (Hooper et al., 2000; Masters and Beyreuther, 1995). Both AGEs and Aβ are present in senile plaques (Wong et al., 2001), and have been shown to upregulate proinflammatory cytokines by RAGE and NFκB-dependent pathways (Schmidt et al., 2000; Yan et al., 1997a, b). This has led to the conclusion that RAGE-Aβ interactions play an important role in the pathophysiology of Alzheimer's disease, and that interference with these processes e.g. by soluble RAGE or novel RAGE antagonists, might be a promising AD therapy (Yan et al., 1998). However, the relevance of RAGE-Aβ binding for Aβ toxicity has been doubted, since Aβ is toxic to neural cell lines and to rat cortical neurons which do not express detectable levels of RAGE (Liu et al., 1997). Since these contradicting data were obtained in different cell lines and cell culture models, it is not clear yet whether the effects of AGEs and Aβ on cellular metabolism are identical or rather different. Thus, we used the (RAGE positive) neuronal cell line SHSY-5Y and systematically compared the effects of AGEs and Aβ on cell viability and various parameters of energy metabolism under identical experimental conditions.

Experimental procedures

Production of AGEs and "fibrillar" Aβ

BSA-AGE was produced by incubation of 1 mM BSA with 1 M glucose at 50°C in PBS (pH 7.4) for 6 weeks. Samples were initially filtered through a 0.2 μm filter and kept sterile during the incubations; the slightly elevated temperature was used to accelerate the reaction and avoid bacterial contamination. Unbound sugars were removed by extensive dialysis with distilled water. AGEs were lyophilized and resuspended in PBS (pH 7.4). The control BSA was incubated under the same conditions, except that glucose was omitted. All preparations were tested for bacterial contamination using the E-toxate test (Sigma), and were found to be below the detection limit of the endotoxin assay. AGE content was characterized by the optical density at 400 nm, the fluorescence at 440 nm (excitation wavelength 370 nm), and by measuring the specific AGE carboxymethyllysine (CML) with a commercially available ELISA (Roche Diagnostics) as described previously (Wagner et al., 2001). Protein content was determined by the Bradford assay, using BSA as standard. The concentrations of the AGE-modified proteins were calculated using the molecular weight of the non-glycated protein. Characteristics of the BSA-AGE preparation were: OD_{400}: 0.56/mg protein, CML: 1.8 nmol/mg protein (0.12 CML per BSA). "Fibrillar" Aβ (1–40) was produced by incubating the peptide (1 mM) in 10 mM sodium phosphate buffer (pH 7.4) under sterile conditions for 4 days at 37°C. Formation of amyloid fibrils was detected by thioflavin T as described previously (Münch et al., 1997).

Peptide synthesis

Aβ (1–40) was produced at the peptide synthesis facility of the University of Würzburg with a Zinsser simultaneous multiple peptide synthesiser using a Fmoc-protection and DIC/HOBt activating chemistry as described previously (Loske et al., 2000). Purity of the peptide was judged by analytical HPLC to be $>98\%$; the correct molecular weight was confirmed by mass spectrometry (MALDI-TOF).

Cell culture

Human neuroblastoma cells (SH-SY5Y) were seeded into 96-well flat bottom tissue culture plates at a density of 3×10^5 cells/ml (for the MTT assay), and for the other assays, into 24-well plates at a density of 6×10^5 cells/ml. Cells were grown in DMEM supplemented with 10% fetal calf serum, streptomycin/penicillin (100 μg/ml; 100 U/ml), 2 mM L-glutamine at 37°C in a humidified atmosphere containing 5% CO_2/95% air. After 48 h, cells were washed with fresh medium and AGEs/Aβ and/or antioxidants at the concentrations indicated were added. RAGE expression was downregulated by daily addition of 8 μg of a RAGE antisense oligonucleotide (5′-AACTGCTGTTCCGGCTGC-3′) per well of the 24 well plate for one week.

RNA-Isolation and detection of RAGE by RT-PCR

1×10^7 SH-SY5Y neuroblastoma cells/flask were grown under same conditions as described. After removal of the medium, cells were washed with PBS and lysed with 1 ml Trizol (Gibco) for 5 min. After centrifugation at 10.000 rpm at 4°C for 10 min, the supernatant was washed with 200 μl chloroform. 400 μl isopropanol was added to the aqueous phase and the mixture was allowed to stand for 10 min. After centrifugation at 10.000 rpm at 4°C for 10 min, the pellet obtained was rinsed with 75% ethanol, dried and dissolved in diethylpyrrocarbonate (DEPC) treated water. Further RNA purification was performed using the Quiagen RNeasy Kit according to the manufacturer's specifications. For reverse transcription of total RNA, the Stratagene RT-PCR Kit (Stratagene Inc., La Jolla, USA) was used. Two primer sets (5′-GAG CCA GAA GGT GGA GCA GT - 3′ and 5′-GAC TGA TTC AGT TCT GCA CG -3′, position 726–646 and 1153–1172; 5′-GGT GCT CAA AAC ATC ACA GCC-3′ and 5′-TCT CAG GGT GTC TCC TGG TC-3′, position 63–84 and 528–547) specific for human RAGE were obtained from ROTH (Karlruhe, Germany). cDNA (5 μl) was added to the following mixture: Taq polymerase buffer (5 μl), each

10 pmol primer (each 1 µl), 25 mM dNPTs (1 µl), sterile water (50 µl). Taq DNA polymerase (0.5 µl, 5 U/µl) was added just before amplification (1 cycle at 95°C for 5 min, 30 cycles at 95°C for 1 min, 55°C for 40 sec, 72°C for 1 min, 1 cycle at 72°C for 10 min. The PCR products (10 µl) were loaded and run on an 1.2% agarose gel and visualized after ethidiumbromide staining by using an UV transluminator.

Detection of RAGE by Western Blot

Cells were lysed with lysis buffer containing 0.5% Triton X100 and 1 mM sodium vanadate. 5 µg protein from each sample was added to loading buffer, heated 5 min at 95°C and loaded on a 8% SDS polyacrylamide gel. After electrophoresis, proteins were blotted from the gel onto a nitrocellulose membrane in a wet tank containing transfer buffer (24.8 mM Tris, 192 mM glycine, 10% methanol). After incubation in 3% milk powder/0.2% Tween 20 overnight at 4°C, and repeated washing with TBS/0.05% Tween 20, the membrane was incubated with a polyclonal goat-anti-RAGE antibody (1:10.000 in TBS/0.1% Tween 20) for 2 h at room temperature, washed four times with TBS for 2 min and incubated with anti-goat IgG peroxidase conjugate (Roche Diagnostics, 1:10.000 in TBS/0.1% Tween 20) for 90 min at room temperature. The membrane was then washed four times for 2 min with PBS, after which detection was performed by chemiluminescence (ECL, Amersham).

MTT assay

The metabolic activity of cells was measured via the intracellular reduction of a soluble (yellow) tetrazolium salt (MTT) to the insoluble (blue) formazan product. The MTT assay measures the NADH dependent reduction of the dye by cytoplasmatic enzymes and to a lesser degree, by the succinate dehydrogenase in mitochondrial complex II (Berridge and Tan, 1993). After incubation of the cells with AGEs/Aβ and the antioxidants, the medium was removed and the cells were washed with PBS. 100 µl of DMEM without Phenol Red and 25 µl of an MTT solution (1.5 mg/ml in PBS) were added to each well, followed by incubation for 4 h. The MTT solution was carefully removed from the wells to avoid loss of formazan crystals, before they were dissolved with 100 µl of DMSO/EtOH 1:1. Absorbance was measured at 550 nm with the reference filter set to 630 nm. Values of untreated controls were set to 100% at each time point. For each experiment, MTT assays were performed in triplicate.

Determination of ATP

ATP was measured by an ATP bioluminescence assay kit (HSII, Roche Diagnostics). Briefly, cells were harvested, resuspended in PBS and lysed according to the manufacturer's instructions. Samples were immediately frozen at − 80°C until analysis with a Berthold 9501 luminometer. ATP concentrations were calculated using a calibration curve of serial ATP dilutions.

Determination of cell numbers

After incubation with Aβ/AGEs and/or test substances, cells were washed with PBS and trypsinized. Cell culture medium was added, and the single cell suspension was centrifuged for 5 min at 800 rpm. Cell numbers were determined with a Schaerfe Casy cell counter equipped with a 150 µm capillary in triplicate experiments. All cells with a size between 9 and 18 µm were considered to be viable.

Determination of lactate concentrations

Lactate concentration in the culture medium was determined in supernatants by the lactate dehydrogenase/glutamate-pyruvate transaminase method. 20 µl cell culture medium and 180 µl distilled water were incubated at 37°C with 200 µl of a reaction mixture containing 250 mM glutamic acid/adjusted to pH 8.9 with NaOH, 2.8 mM NAD, 0.275 U/ml glutamate-pyruvate transaminase and 2.75 U/ml lactate dehydrogenase for 1 h, after which the absorbance at 340 nm

was measured. Lactate concentrations were calculated by comparison with a calibration curve using lactate standards dissolved in cell culture medium.

Determination of glucose concentrations

Glucose concentration in the culture medium was measured by conversion of glucose to gluconate by glucose-oxidase (Trinder, Sigma). Briefly, 5 µl of the culture supernatants were diluted in 45 µl distilled water and 50 µl of test reagent was added. After 10 min of incubation at room temperature, the absorbance at 550 nm was measured. Glucose concentrations were calculated by comparison with a calibration curve using glucose standards dissolved in cell culture medium.

Statistics

Statistical analysis of the results was carried out by ANOVA using the Statview statistics program. Statistical significance was established at a p-value < 0.05 (*) or < 0.01 (**), respectively.

Results

Expression of RAGE in SH-SY5Y neuroblastoma cells

AGEs and Aβ are both structural components of the senile plaques in the AD brain, bind to a common receptor, the receptor for advanced glycation endproducts (RAGE) (Schmidt et al., 1994; Yan et al., 1997a, b), and are neurotoxic by mechanisms involving oxidative stress (Behl and Sagara, 1997; Smith et al., 1995; Vitek et al., 1994). Since AGEs and Aβ have been proposed to act through a common receptor, RAGE, we compared their effects on cell viability, glucose consumption, lactate production and ATP level under identical experimental conditions in the same cell line. The human SH-SY5Y neuroblastoma cell line was chosen for this purpose, since it has been used previously as a neuronal cell model for the investigation of AGE and Aβ toxicity (Lambert et al., 1994; Loske et al., 1998). RAGE was detectable on the mRNA and protein level. Furthermore, incubation of the cells with a RAGE antisense oligonucleotide for a week led to a lower expression of the two RAGE isoforms (Fig. 1).

Effects of AGEs and Aβ on cell viability and ATP levels

In the initial set of experiments, dose-dependent effects of AGEs and Aβ on cell viability were compared. Similar to our previous experiments (Loske et al., 1998), BSA-AGE (used as a model-AGE) and Aβ were applied in concentrations of 50 and 100 µM. Cell viability was determined by the MTT assay, which measures metabolic activity (intracellular NADH concentration) of the cell, and by direct cell counting. Both AGE and Aβ led to a significant reduction in MTT activity in a dose-dependent manner. In contrast, only AGE, but not Aβ, reduced the actual number of counted viable cells in a dose-dependent manner, e.g. to $65 \pm 17\%$ at 100 µM AGE (Fig. 2).

In a second set of experiments, the dose-dependent effects of AGEs and Aβ on ATP level were determined. Since AGEs caused a reduction of cell number compared to the untreated conrol (Fig. 2; Table 1), all energetic parameters (the molar amount of ATP, glucose and lactate) shown in the following experiments were normalized to the number of cells present at $t = 24$ h. AGEs significantly decreased ATP concentration (e.g. to $58 \pm 16\%$ at 100 µM

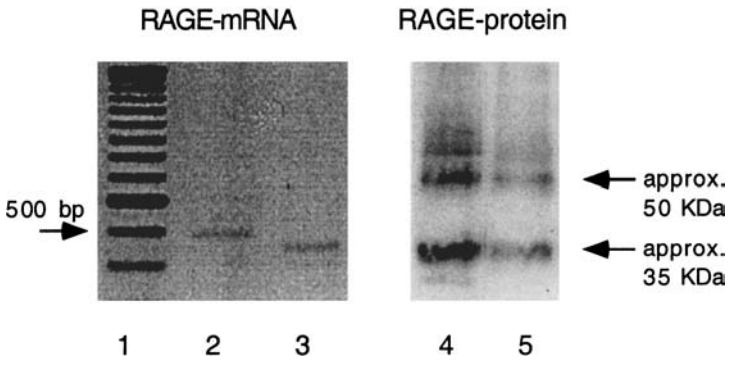

Fig. 1. Expression of RAGE in SH-SY5Y neuroblastoma cells and its specific downregulation by antisense oligonucleotides. SH-SY5Y neuroblastoma cells express RAGE, which can be detected both on the mRNA (lane 1: 100 bp ladder; lane 2 and 3: 484 bp and 446 bp RAGE PCR products) and on the protein level (lane 4: constitutive expression of RAGE, lane 5: downregulated expression of RAGE when cells were preincubated with a RAGE antisense nucleotide for 1 week)

BSA-AGE) in a dose-dependent manner (Fig. 3, lane 8). Fibrillar Aβ also decreased ATP levels, e.g. to appromimately $78 \pm 12\%$ at 100 μM Aβ compared to the untreated control (Fig. 3, lane 4). 100 μM "fresh" monomeric (non-fibrillar) Aβ and non-modified BSA had no effect on ATP levels (Fig. 3, lanes 1, 2, 5 and 6).

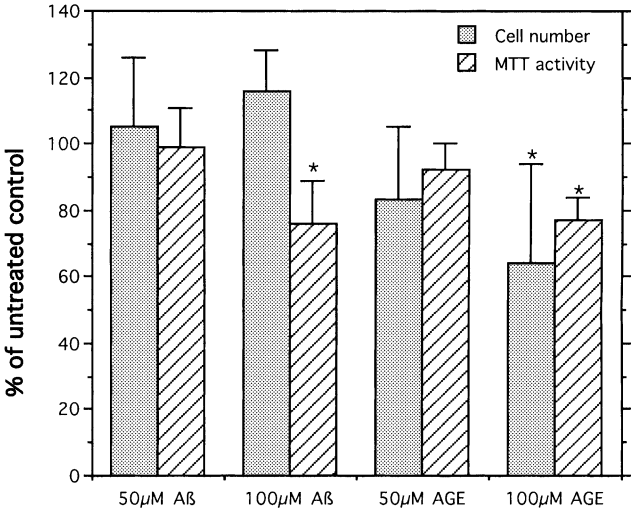

Fig. 2. Changes in cell number and MTT reduction activity after incubation of cells with Aβ and AGEs. Cells were incubated with 50 μM and 100 μM BSA-AGE or 50 μM and 100 μM Aβ for 24 h. AGEs and Aβ decreased MTT reduction activity, but only AGEs had an effect on cell numbers. Data are taken from 2 separate experiments (each done in triplicate) and are expressed as mean ± SD (*/** indicates significance ($p < 0.05/0.01$) in comparison to the untreated control at $t = 24$ h)

Table 1. Cell number, glucose uptake and lactate production after addition of AGEs or fibrillar Aβ

	Cells/well at t = 24 (start: 1×10^6)	Glucose consumption (pmol/cell)	Lactate production (pmol/cell)
No addition	$1.80 \pm 0.30\times10^6$	0.65 ± 0.08	1.42 ± 0.39
100 μM BSA-AGE	$1.15 \pm 0.51\times10^6$	1.95 ± 0.36	8.64 ± 0.42
100 μM fibrillar Aβ	$2.10 \pm 0.10\times10^6$	1.08 ± 0.13	1.22 ± 0.41

Effects of AGEs and Aβ on glucose consumption and lactate production

The observed decrease in ATP level caused by both AGEs and Aβ suggests either increased ATP consumption or decreased ATP production, which could be caused by the inhibition of glycolysis or mitochondrial respiration. To investigate and compare further effects of Aβ- and AGE-induced changes in glucose metabolism, we determined total glucose consumption and lactate production after a time period of 24 h.

Glucose consumption: Glucose consumption (assayed by glucose uptake from the medium) was determined indirectly by measuring the amount of glucose remaining in the culture supernatant after incubation with AGEs or Aβ after 24 h. With 100 μM BSA-AGE, a 3-fold increase in glucose consumption could be observed by comparison to an equimolar concentration of non-modified

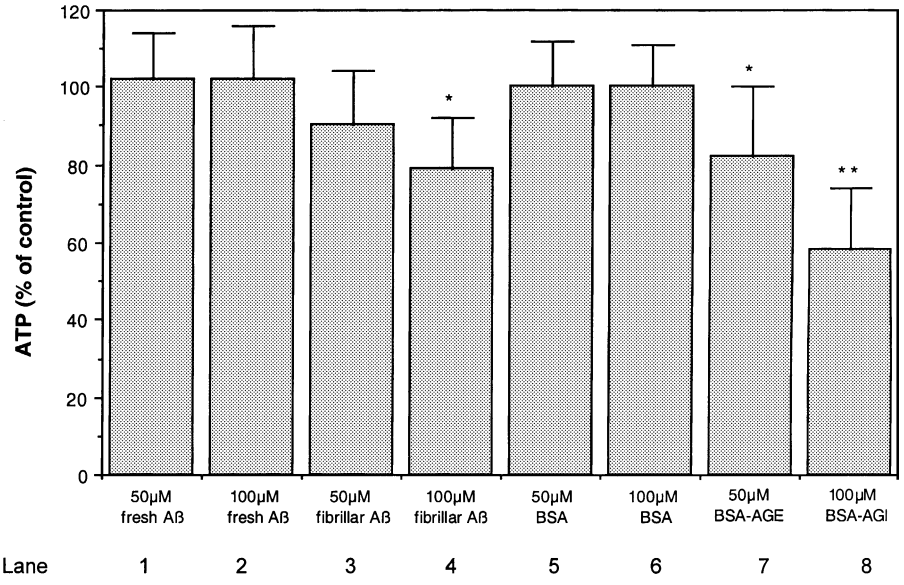

Fig. 3. Changes in ATP level after incubation with AGEs and Aβ. Fibrillar Aβ and BSA-AGE decrease ATP level in SH-SY5Y neuroblastoma cells compared to untreated controls (lanes 3, 4/7, 8). Freshly dissolved Aβ and unmodified BSA had no effect (lanes 1, 2 and 5, 6). Cells were incubated with 50 μM and 100 μM "fibrillar" Aβ for 24 h. Data are taken from 3 separate experiments (each done in duplicate) and are expressed as mean ± SD (* indicates significance ($p < 0.05$) in comparison to the negative control (no AGE or unmodified protein added))

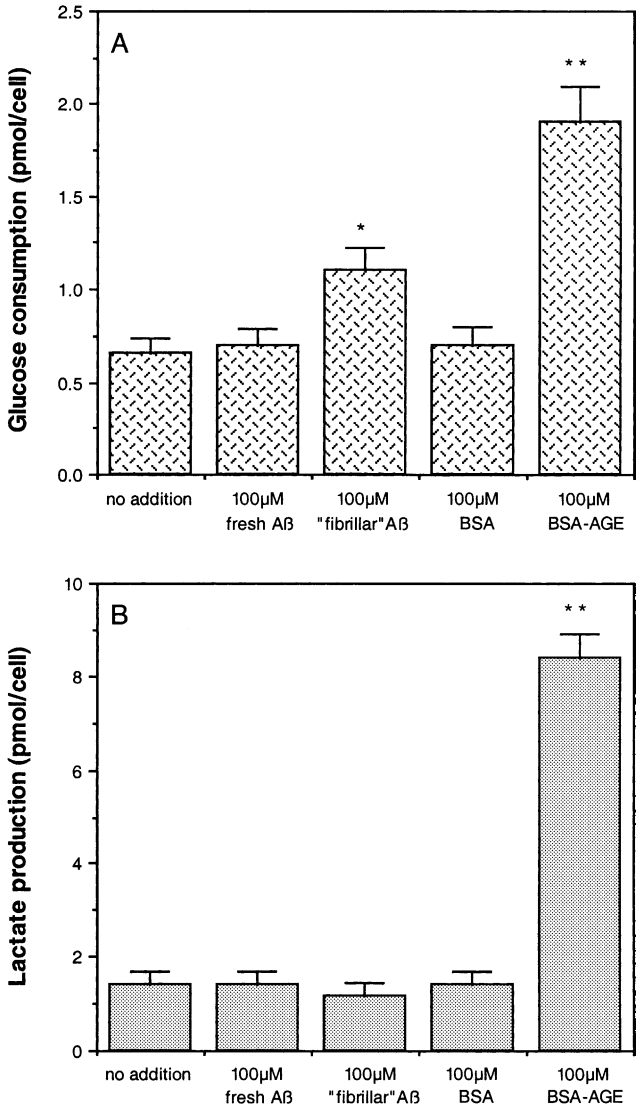

Fig. 4. Changes in glucose consumption and lactate production caused by AGEs and Aβ. Fibrillar Aβ and BSA-AGE increase glucose consumption in SH-SY5Y neuroblastoma cells compared to untreated control over time period of 24 h. However, only BSA-AGE showed a dramatic increase in lactate production. Data are taken from 4 separate experiments and are expressed as mean ± SD ($^{*}/^{**}$ indicates significance ($p < 0.05/0.01$) in comparison to the negative control (left column)

BSA (Fig. 4A, Table 1). 100 µM "fibrillar" Aβ also significantly increased glucose consumption of the cells after an incubation time of 24 h (Fig. 4A). In contrast, fresh (non-fibrillar) Aβ had no influence on glucose consumption.

Lactate production: Lactate production was increased 6-fold in cells incubated with 100 µM BSA-AGE (Fig. 4B, Table 1). Very unexpectedly, 100 µM "fibrillar" Aβ did not lead to an increase in lactate production (Fig. 4B), suggesting a

quite different mode of action of AGEs and Aβ in terms of anaerobic glucose metabolism.

Discussion

AGEs are produced by nonenzymatic glycation and oxidation reactions between carbohydrate-derived carbonyl compounds and protein amino groups and are cytotoxic by a mechanism involving reactive oxygen and nitrogen species (Loske et al., 1998; Takeuchi et al., 2000). Since AGEs accumulate on β-amyloid plaques in the brains of AD patients over a period of 30 years, they might aggravate Aβ-mediated oxidative stress, cell damage, functional loss and even neuronal cell death in the AD brain via RAGE receptor dependent mechanisms. Here, we have determined the effects of AGEs which have been extensively modified by AGEs to reflect the fact that the age of a senile plaque *in vivo* (up to 30 years) is many times higher than the half-life of an AGE-modified plasma protein (1–3 months).

Both AGE and Aβ applications led to a significant reduction in cell viability in a dose-dependent manner, as determined by the MTT assay. In addition to the Thioflavin T assay (see methods), a decrease in MTT reduction activity confirms the presence of fibrillar (''toxic'') Aβ oligomers in our Aβ preparation. However, the MTT assay, which has been widely used to propose the direct neurotoxicity of Aβ in the past, does not exactly reflect the number of cells or their metabolic activity when they are exposed to Aβ. For example, it was shown that Aβ inhibits MTT reduction directly by facilitating exocytosis of MTT formazan rather than affecting cell viability or glucose metabolism (Abe and Saito, 1998). Thus, direct counting of viable cells appears to be the more reliable assay to measure the effects of both RAGE ligands on cell viability. When the number of cells was compared after 24 h, only AGE, but not Aβ, reduced the actual number of cells in a dose-dependent manner compared to untreated control cells, suggesting a quite different effect of the two RAGE ligands on cell viability. However, it should be noted that 100 μM BSA-AGE did not lead to a reduction of total cell number, which was still above the number seeded at the start of the experiment (Table 1). Thus, the effect of BSA-AGE on SH-SY5Y cells can be considered rather as an inhibition of cell proliferation than a direct neurotoxicity.

It has been shown previously that intracellular ATP concentration is compromised during the first 24-h exposure of neurons to Aβ (Zhang et al., 1996). In this study, we show that both Aβ and AGEs lead to a dose-dependent decrease in ATP levels. Since ATP is important for many neuronal functions including the maintenance of the $Na+/K+$ gradient, low ATP levels in neurons of affected patients (caused either by Aβ or AGE) might directly influence synaptic transmission and create the clinical picture of dementia.

Glucose consumption, which was measured as the uptake of glucose from the medium over 24 h, was increased by both 100 μM AGE and 100 μM ''fibrillar'' Aβ. Increased glucose consumption induced by Aβ has been shown in brain vascular endothelial cells (Preston et al., 1998). Such an initial increase in glucose uptake can be observed in a cellular model system when

mitochondrial function is impaired by a blockade of the electron transport chain with rotenenone (Bashan et al., 2003). Contrary results have been described for cortical rat neurons, where Aβ decreased glucose transport into the cell (Mark et al., 1997). This discrepancy could be explained by the fact that glucose consumption might be higher to satisfy increased energy demands in the early stages of Aβ exposure but decreases later when irreversible inactivation of redox-sensitive enzymes such as aconitase occurs (Gardner et al., 1995).

A very unexpected and striking observation was the fact that AGEs but not Aβ caused a massive (6 fold) increase in lactate production. These data suggest that AGEs may shuttle pyruvate into anaerobic glycolysis, allowing NAD to be regenerated as hydrogen acceptor. This occurs when glucose flux through the Krebs cycle and subsequent oxidative phosphorylation are impaired. One possible explanation for increased lactate production would have been the inactivation of pyruvate dehydrogenase. Although such a process has not been shown for AGEs so far. However, it is known that AGEs produce oxygen free radicals (Wautier et al., 2001) and lipid peroxidation products such as acrolein and hydroxynonenal (Gasic-Milenkovic et al., 2003). Furthermore, it is known that hydroxynonenal inactivates pyruvate dehydrogenase (PDH) (Humphries et al., 1998), which might suggest that AGEs could lead to an inactivation of PDH by a similar mechanism.

Very interestingly, when the ratio of lactate production vs. glucose consumption was calculated, it was quite different in AGE vs. Aβ treated cells.

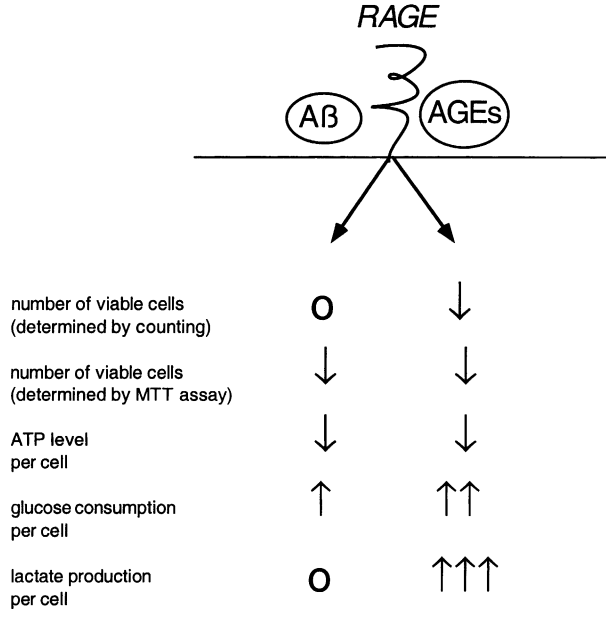

Fig. 5. Comparison of the effects of Aβ and AGEs on cell viability and energetic parameters. Differential effect of Aβ and AGEs, which are proposed to transmit their signal through a common receptor (RAGE), on cell viablity and energetic parameters are shown in comparison (o: = ± 20% difference, ↑: increase + 20–50%, ↑↑: + 50–100%, ↑↑↑: + > 100%, ↓: decrease – 20–50%, ↓↓: – 50–100%)

During treatment with 100 μM BSA-AGE, more than 4 molecules of lactate were produced per molecule of glucose consumed. Although these preliminary data must be clarified by direct determination of individual amino acid, our data suggest that fuel sources other than glucose (e.g. amino acids) are converted into lactate. If amino acids are used for energy production, glutamate (produced from glutamine) would be converted into α-ketoglutarate and used or further oxidation. Although this is quite a speculative hypothesis, consumption of glucogenic amino acids might explain the fact that increased levels of ammonia are released from the brain of AD patients in early stages of the disease compared to aged matched non-demented controls (Hoyer et al., 1990). However, with 100 μM Aβ, the lactate/glucose ratio was less than 2, suggesting that amino acid utilization is not up-regulated and that glucose is rather used for antioxidant defenses, e.g. via the pentose phosphate pathway to regenerate NADPH for the reduction of oxidized glutathione (Kussmaul et al., 1999).

In summary, AGEs and Aβ both lead to decreased ATP levels in neurons despite an increased glucose uptake and consumption. However, only AGEs lead to a dramatic increase in lactate production, suggesting that the metabolic consequences of interactions of Aβ and AGEs with cells are not transmitted via identical pathways, mediated by the RAGE receptor. In addition to alternative binding sites on RAGE for AGEs and Aβ, which subsequently leads to alternative transmission of downstream signals, these differential signals could also be transmitted via alternative AGE receptors such as the macrophage scavenger receptors AGE R1, AGE R2 or AGE R3 (Araki et al., 1995; Li et al., 1998; Thornalley, 1998; Vlassara et al., 1995) (Fig. 5).

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