Research Article

Insulin Promotes Survival of Amyloid-Beta Oligomers Neuroblastoma Damaged Cells via Caspase 9 Inhibition and Hsp70 Upregulation

M. Di Carlo,[1] P. Picone,[1, 2] R. Carrotta,[3] D. Giacomazza,[3] and P. L. San Biagio[3]

[1] *Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", Consiglio Nazionale delle Ricerche, Via U. La Malfa, 153, 90146 Palermo, Italy*
[2] *Dipartimento di Chimica e Tecnologie Farmaceutiche, Università di Palermo, Via Archirafi, 36, 90146 Palermo, Italy*
[3] *Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Via U. La Malfa, 153, 90146 Palermo, Italy*

Correspondence should be addressed to M. Di Carlo, di-carlo@ibim.cnr.it

Alzheimer's disease (AD) and type 2 diabetes are connected in a way that is still not completely understood, but insulin resistance has been implicated as a risk factor for developing AD. Here we show an evidence that insulin is capable of reducing cytotoxicity induced by Amyloid-beta peptides (A-beta) in its oligomeric form in a dose-dependent manner. By TUNEL and biochemical assays we demonstrate that the recovery of the cell viability is obtained by inhibition of intrinsic apoptotic program, triggered by A-beta and involving caspase 9 and 3 activation. A protective role of insulin on mitochondrial damage is also shown by using Mito-red vital dye. Furthermore, A-beta activates the stress inducible Hsp70 protein in LAN5 cells and an overexpression is detectable after the addition of insulin, suggesting that this major induction is the necessary condition to activate a cell survival program. Together, these results may provide opportunities for the design of preventive and therapeutic strategies against AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is characterized by neuronal cell loss and progressive accumulation of neurofibrillary tangles (NFT) in neurons, and amyloid fibers in neuritic (senile) plaques and in the walls of blood vessels [1]. This physiopathological disorder causes a gradual loss of memory and judgment. As the disease progresses, AD patients experience changes in personality, behavior, and social interactions.

Amyloid beta peptide (A-beta) is the major component of amyloid plaques in the brain of individuals affected by AD. The formation of the plaques is due to an overproduction of A-beta through enzymatic cleavage of the larger amyloid protein precursor (APP). While the monomeric A-beta is not neurotoxic, under specific conditions it is able to misfold from its soluble form into small oligomers and highly ordered fibrillar aggregates. This phenomenon is known as the aggregation of proteins and it is a characteristic feature of several neurodegenerative diseases [2]. Although neuronal

degeneration occurs near the amyloid plaques, some studies have suggested that intermediates such as protofibrils or simple oligomers are also involved in AD pathogenesis and even appear to be the more dangerous species in the onset of the pathology [3].

Genetic researches have demonstrated that only a small fraction (about 5%) of all AD cases is caused by inherited alterations with precocious symptom appearance (before age 65) [4]. The early AD onset is, in most cases, originated by mutations in three genes: APP, PSEN1, and PSEN2. As a result, a large amount of A-beta toxic fragments is produced and deposited as plaques. The great majority of all AD cases is sporadic in origin, with old age (>65) as the main risk factor.

The "amyloid cascade hypothesis" has tried to explain the origin of the disease. This theory postulates that the deposition of A-beta is the origin of the pathology and that cell loss, dementia, and vascular damage are strictly linked with this deposition. This hypothesis can give an explanation for the AD early onset but it is not viable for the AD sporadic form. Indeed, patients affected by AD late-onset very rarely

present APP, PSEN1, and PSEN2 gene mutations. Further, the presence of the plaques can also be observed in the elderly without AD development [5].

Type 2 diabetes is associated to a reduced ability of insulin to stimulate glucose utilization (insulin resistance). It has been recently recognized that AD is closely linked to the diabetes mellitus in a way that it is still unclear and a deficiency in the glucose metabolism can be considered a risk factor in the sporadic AD onset [6–8]. Further some diabetes drugs appear to slow the cognitive decline associated with AD [9]. It has been also demonstrated that extracellular injection of insulin is able to protect neurons against A-beta induced cell death [10].

It has been reported [11] that insulin can protect cultured rat neurons against A-beta induced toxicity. Experimental data [12] have demonstrated that A-beta competes for binding of insulin to its receptor. This results in a decrease in autophosphorylation of the insulin receptor. However, it is not understood if this effect was originated through the binding of A-beta to insulin or directly to the insulin receptor. By using mature cultures of hippocampal neurons it was found that A-beta soluble oligomers (also known as ADDLs) caused loss of the activation of insulin receptors (IR) on the neuronal surface, and this event was linked to disruption of insulin signaling [13]. Other studies indicate that insulin, interacting with A-beta, inhibits its fibrillar growth as shown in a cell-free assay and in the cell surface of human brain pericytes [10] reducing the A-beta toxic effect.

Recently, in two different model systems, sea urchin embryo and neuroblastoma cell line, it has been demonstrated that A-beta oligomers are more toxic than larger and highly structured fibrils [14, 15]. In particular, oligomers and fibrils, involved in the neurodegenerative process are able to induce two different apoptotic pathways [15]. The authors postulate that the large fibrils, remaining on the extracellular space and obstructing the membrane functional channels, can induce an extrinsic programmed cell death pathways through the activation of caspase 8 only. On the contrary, the small oligomers are able to penetrate into the cells. They, damaging the mitochondria, cause a release of the cytochrome C that, in turn, induces activation of the intrinsic apoptotic pathway by the intervention of the caspase 9.

The present study is addressed to investigate whether insulin is able to protect cells by A-beta oligomers toxicity, through inhibition of specific apoptotic pathways activation.

2. Materials and Methods

2.1. Cell Cultures and Treatments. LAN5 human neuroblastoma cell lines were plated onto 96-well plates at a density of 6×10^4 per well and cultured with RPMI 1640 medium (CELBIO) supplemented with 10% fetal bovine serum (FBS) (GIBCO) and 1% antibiotics (50 μ/mL penicillin and 50 μg/mL streptomycin) and antimycotics (SIGMA). Cells were maintained in humidified 5% CO_2 atmosphere at 37°C.

In order to obtain the oligomers, recombinant Aβ42 (rAβ42) was dissolved in 0.01 M TrisHCl buffer at pH 7.2 [14] and insulin (SIGMA) in HEPES buffer at pH 8.2.

Cells were treated with two different concentrations 25 and 40 μM of oligomeric rAβ42 [14] for 1 h. After this treatment rAβ42 was removed and the cell were incubated without or with insulin at different concentrations (50, 100, 200 μM) in medium serum free at 37°C for 20 h. For successive experiments we selected the A-beta oligomer and insulin concentrations at 40 and 100 μM, respectively. In caspase assays, the incubation time with insulin was 4 h. The treated cultured cells and the controls were morphologically analysed by microscopy inspection using an Axioscop 2 microscope (Zeiss, USA) or used for specific assays. Depending on the experiments we also utilized, as controls, both the untreated cells and the cultured cells with buffers at the concentration utilized to dissolve rAβ42 or insulin.

2.2. Determination of Cell Viability. Cell viability was measured by MTS assay (PROMEGA). MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfopheyl)2H-tetrazolium] was utilized according to the manufacturer's instructions. After treatments of the cells, 20 μL of the MTS solution were added to each well, and the incubation was prolonged for 4 h at 37°C, 5% CO_2. The absorbance was read at 490 nm on the Microplate reader ^{wallac}Victor^2 1420 MULTILABEL COUNTER (Perkin Elmer). Results were expressed as the percentage of MTS reduction as regard to the controls.

2.3. Apoptosis Assay. TUNEL assay was performed according to the manufacturer instructions (PROMEGA). Briefly, cells untreated or after treatment with rAβ42 alone or with insulin were fixed with 4% paraformaldehyde in PBS for 30 min. Then they were washed with PBS, permeabilized with 0.2% Triton X-100 in PBS for 5 min, rinsed with PBS and incubated with TUNEL reaction mixture (enzyme, nucleotides) in an humidified atmosphere at 37°C for 1 hour. Staining was obtained by using a peroxidase substrate, hydrogen peroxide and the stable chromogen, diaminobenzidine (DAB). After this procedure, samples were rinsed three times with PBS and analysed under Zeiss Axioscop microscope.

2.4. Mitochondrial and Nuclear Staining. One vial of Mito red was dissolved in DMSO according to the manufacturer instructions (SIGMA). Living cells were incubated with 20 nM Mito red for 5 min and red staining indicates that the mitocondrial is active. For nuclear staining, cells were incubated in the Hoechst 33258 (5 μg/mL) for 30 min and intense blue staining indicates nuclear fragmentation. Nuclear morphology and mitochondrial activity were analyzed by microscopic inspection using a Leica DHL fluorescent microscope at excitation/emission wavelengths of 350/450 nm, respectively.

2.5. Caspase Assays. Caspase-8, -9, and -3 activities in cells were measured using commercially available luminescent assays (caspase-Glo 8, caspase-Glo 9 and caspase-Glo 3/7 assay systems, PROMEGA). LAN5 cells were treated with oligomers alone or with insulin. LAN5 cells untreated or
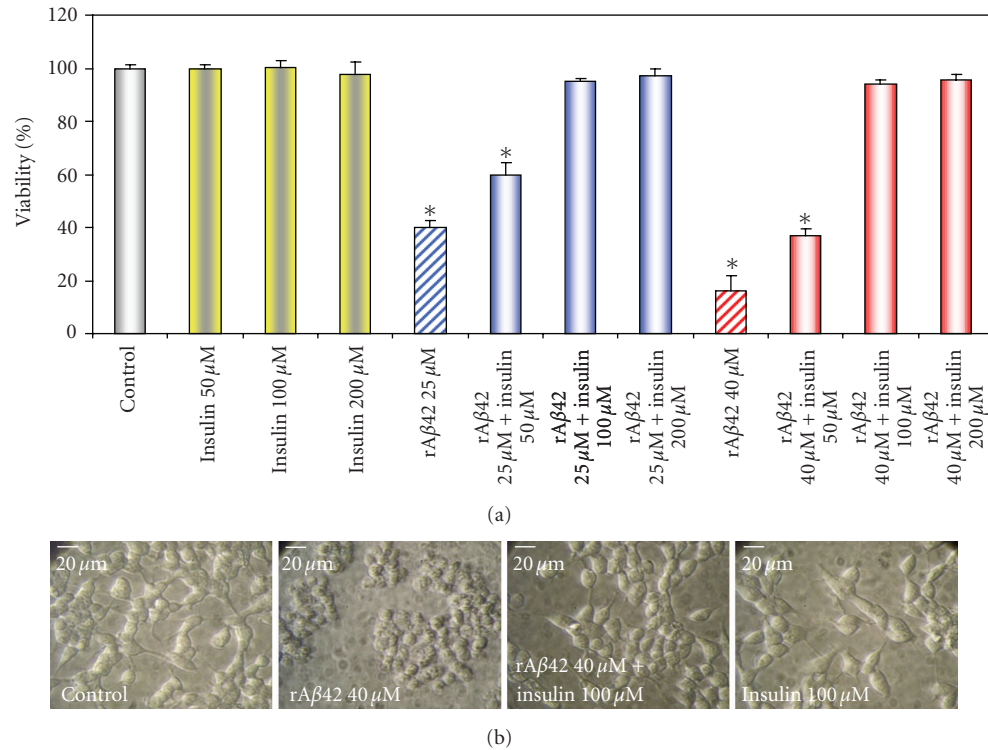
(a)

(b)

FIGURE 1: Dose dependence of protective effect of insulin against rAβ42 oligomers induced cell death. (a) LAN5 neuroblastoma cells were untreated (control) or incubated with insulin 50 or 100 or 200 μM of insulin alone or after incubation with 25 or 40 μM of rAβ42. After incubation treated and untreated cells were submitted to viability MTS assay. Cell viability is significantly lower in rAβ42 25 μM *P < .01, rAβ42 25 μM + insulin 50 μM *P < .01 versus control, whereas it is not significantly lower with rAβ42 25 μM + insulin 100 μM versus control. Cell viability is significantly lower in rAβ42 40 μM *P < .003, rAβ42 40 μM + insulin 50 μM *P < .003, whereas it is not significantly lower with Aβ 40 μM + insulin 100 μM versus control. Percentage of viability is referred to control, the data are the mean ± SD of three separate experiments. (b) Representative morphological images of LAN5 untreated cells (control), treated with rAβ42 40 μM, rAβ42 40 μM, and insulin 100 μM, insulin alone 100 μM. Bar 20 μm.

treated with insulin alone were used as control. Caspase reagent specific for each kit was added directly to the cells in white 96-well plates and after mixing, they were incubated for 15–30 min before recording luminescence with ʷᵃˡˡᵃᶜVictor² 1420 MULTILABEL COUNTER (Perkin Elmer) apparatus. The caspase activator assay was performed using 50 μM vinblastine (SIGMA).

2.6. Protein Extraction and Western Blotting. Total proteins were prepared by dissolving in solubilizing buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% Triton X-100, 2 mM PMSF, 10 μg/mL protease inhibitor, 1mM Na$_3$VO$_4$, and 1mM NaF) LAN5 cells untreated (control) or treated with oligomers, alone or with insulin. Protein samples (20 μg) were electrophoretically separated using 10% SDS-PAGE gel and transferred onto nitrocellulose filters for immunoblotting. After blocking in 3% BSA in TBST, the Western blot was incubated with antiphosphorylated Hsp70 (Hsp70) (1 : 1000; Cell Signalling) or anticonstitutive Hsp70 (Hsc70) (1 : 500; Cell Signalling) or anti β-actin (1 : 1000). Primary antibodies were detected using the ECL chemiluminescence kit (Amersham) according to the manufacturer's instructions and using secondary antibodies conjugated to horseradish

peroxidase (1 : 1500; Amersham). Band intensities were analyzed with a gel documentation system (BioRad). Hsp70 and Hsc70 expression were adjusted to β-actin expression. The protein levels were expressed as densitometry.

2.7. Statistical Analysis. All experiments were repeated at least three times. Each experiment was performed in triplicate. The results are presented as mean ± SD. Statistical evaluation was conducted by ANOVA, followed by Student's t-test for analysis of significance. P < .05 was considered statistically significant.

3. Results

3.1. Insulin Protects Neuroblastoma Cells against rAβ42 Oligomers Toxicity. As a first step to evaluate the amount of insulin necessary to interfere with cell damage induced by A-beta oligomers, a dose-response study was performed. LAN5 neuroblastoma cells were treated before with different amounts of rAβ42 oligomers (25, 40 μM) and then with different insulin concentrations (50, 100, 200 μM).

As shown in Figure 1(a), cells treated with rAβ42, 25 μM or 40 μM showed a mortality of about 60% and 85%,

(a)

(b)

(c)

(d)

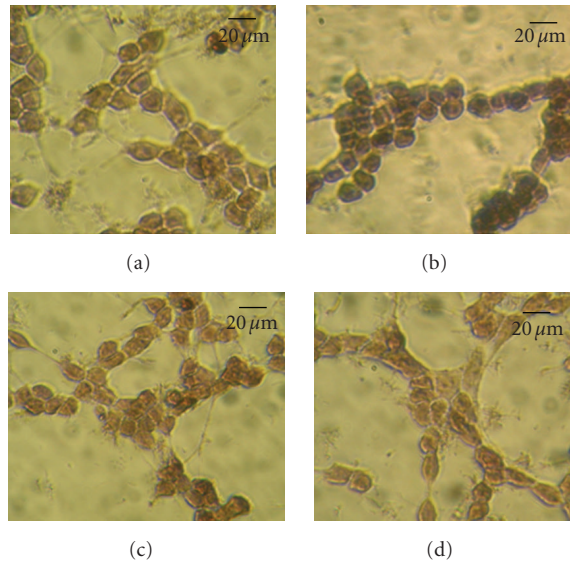FIGURE 2: Protective effect of insulin against rAβ42 oligomers induced apoptosis. LAN5 untreated cells as control (a), treated with rAβ42 oligomers (40 μM) for 1 h (b), with rAβ42 (40 μM) for 1 h and with insulin (100 μM) for 20 h (c), insulin alone (100 μM) for 20 h (d) were fixed and put through TUNEL assay. Bar 20 μm.

respectively, if compared to the control. When insulin was added, in the sample previously treated with oligomers, a recovery of cell viability of about 20% for the minor concentration and 100% for the higher concentration was observed, indicating that insulin plays a protective effect against A-beta toxicity (Figure 1(a)).

Moreover, to visualize the results obtained by the viability assay, the morphological effect compared to the corresponding controls was examined by microscopic inspection. Neurons treated with rAβ42 oligomers at different degeneration steps were observed and some representative images are shown in Figure 1(b). Morphological changes resulted in a reduction of the cellular body, neuritis, and cell numbers. Cells treated with rAβ42 oligomers and insulin appeared to recover the regular morphology of neurons.

3.2. Insulin Reduces Apoptosis Induced by rAβ42 Oligomers.
In apoptosis a biochemical cascade activates proteases that destroy biomolecules required for cell survival. During this process the cytoplasm condenses, organelles aggregate, chromatin condenses, and nucleus fragments [16]. After cell treatment with rAβ42 oligomers, we detected in the survived cells morphological modifications, typical hallmarks of the apoptosis process. In particular granules, resembling apoptotic bodies, were observable (data not shown). To investigate if insulin can revert this effect, LAN5 cells were put through to the TUNEL assay after treatment with oligomers alone or with oligomers and insulin. An intense brown nuclear staining is visible in the cells treated with rAβ42 (Figure 2(b)), indicating that the apoptotic process has been triggered, whereas no staining is detectable in the cells treated with oligomers and insulin (Figure 2(c)), as seen for the control cells (Figures 2(a) and 2(d)).

3.3. Insulin Inhibits Intrinsic Apoptotic Pathway Induced by rAβ42 Oligomers.
The major executioners in the apoptotic program are proteases known as caspases. In some forms of apoptosis, the extrinsic apoptotic pathway is initiated by activation of caspase 8 after death receptor binding; in other forms, activation of the intrinsic apoptotic pathway is initiated by signaling molecules, recruited by mitochondria. They lead a release of cytochrome C from mitochondrial matrix to cytoplasm where it binds to Apaf-1 protein to form the apoptosome that activates caspase 9 [16]. Both these pathways are able to activate the executrix caspase-3 involved in the final part of death process. To identify in which rAβ42-oligomer induced apoptotic pathway insulin interferes, we performed caspase 8 and caspase 9 luminometric assays. A caspase activator was employed as a control. According to previous results, no activation of capsase 8 by oligomers stimulus occurred [15] and the same result for insulin stimulus was obtained (Figure 3(a)). Instead, as can be seen in Figure 3(b), A-beta oligomers activate caspase 9 and this activation is noticeably reduced by the presence of insulin, indicating, once again, that insulin is able to produce a positive protective effect. In order to confirm this result, LAN5 cells treated as described above were put through to caspase 3 assay, the typical executrix caspase. As expected, in the LAN5 cells treated with oligomers and insulin a reduction in the caspase 3 activation with respect to the A-beta oligomer treated cells was detected (Figure 3(c)). These results confirm that A-beta oligomers induce intrinsic apoptotic pathway and the insulin is able to down-regulate caspases 9 and 3 activation.

3.4. Insulin Recovers Mitochondrial Respiratory Activity Reduced by rAβ42 Oligomers.
Caspase 9 activation is considered an event subsequent to mitochondrial damage. In order to confirm that a dysfunction of this organelle occurred after A-beta stimulus and this dysfunction is replaced or avoided by insulin treatment, we tested the metabolic activity of the mitochondria by using Mito red, a specific dye that permits to measure the respiratory activity. Once the dye is introduced into the cells, it freely diffuses through the outer mitochondrial membrane and enters exclusively into the mitochondrial matrix of the metabolically active mitochondrials. The red staining, shown in Figure 4, indicates mitocondrial activity. Moreover, the nucleus integrity was visualized by staining the samples with the specific Hoechst dye. In Figure 4 an intense red staining, comparable to the control cells, was observed in cell treated with A-beta oligomers and insulin and no intense blue staining, indicating nuclear fragmentation, was observed. In contrast, absence of red staining is visible in the cells treated with A-beta oligomers alone, while an intensive blue staining is evident, stating the presence of DNA nicks, a typical apoptosis hallmark. This analysis confirms that the rAβ42 induced degeneration, via mitochondrial damage, has been counteracted by insulin addition.

3.5. Modulation of Stress Response Activation.
Hsp70 has a negative regulatory role in apoptosis and is activated, by phosphorylation process, in response to a variety of
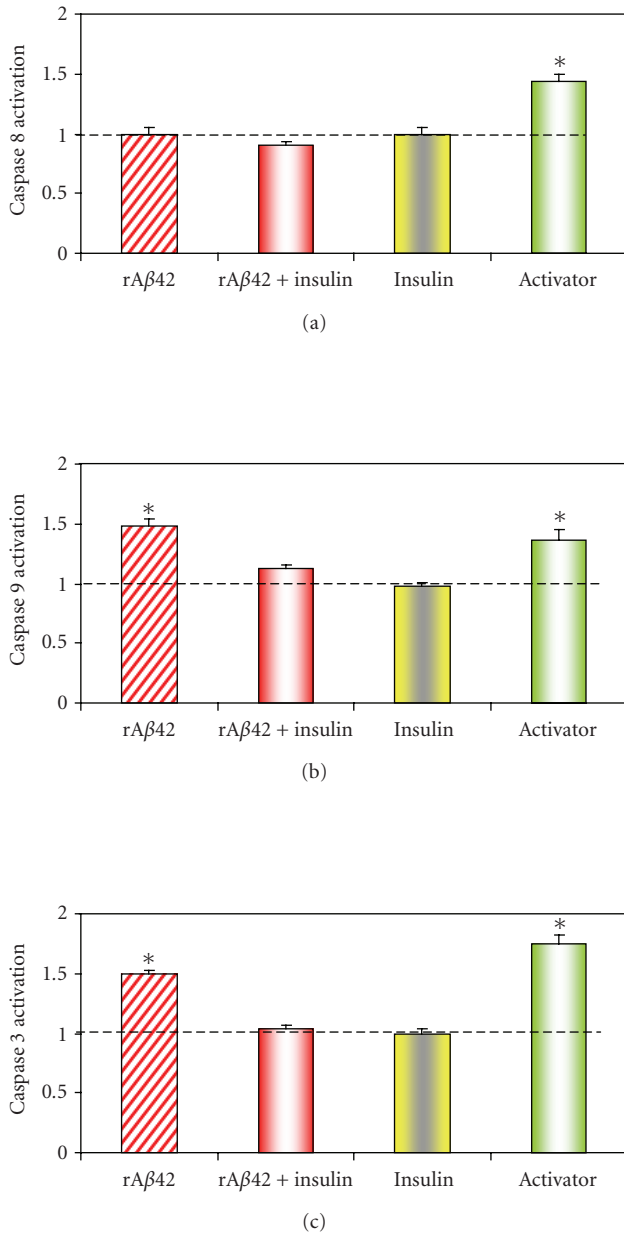
(a)

(b)

(c)

FIGURE 3: Effects of insulin against rAβ42 oligomers on caspase 9 and 3 activation. LAN5 were untreated (control), treated with rAβ42 oligomers (40 μM) for 1 h (rAβ42), rAβ42 oligomers (40 μM) for 1 h and insulin (100 μM) for 4 h (rAβ42 + insulin), insulin alone (100 μM) for 4 h (insulin), 50 μM Vinblastine for 4 h (activator) and submitted to caspase 8 (a), caspase 9 (b), and caspase 3 (c) luminescent assays. Data expressed as percentage of the corresponding control. The dashed line shown in the histogram corresponds to a control culture. The activation of caspase 8 (a) is significantly high with activator *$P < .02$, whereas it is not significantly high in rAβ42 versus control. The activation of caspase 9 (b) is significantly high in rAβ42 *$P < .01$ and in the activator *$P < .05$, whereas it is not significantly high in rAβ42 + insulin versus control. The activation of caspase 3 (c) is significantly high in rAβ42 *$P < .01$ and in the activator *$P < .01$, whereas it is not significantly high in rAβ42 + insulin versus control. Values are referred to control, the data are the mean ± SD of three separate experiments.
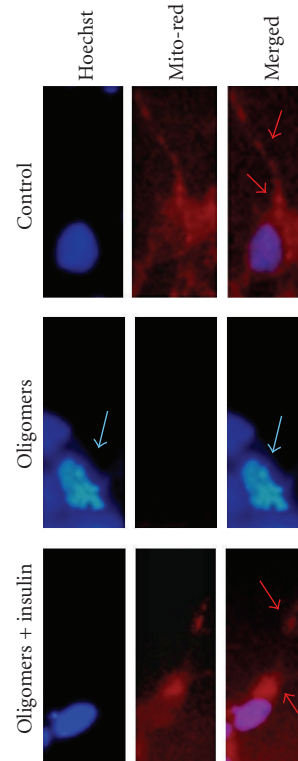

FIGURE 4: Insulin recovers mitochondrial respiratory activity, reduced by rAβ42 oligomers. LAN5 were untreated (control), treated with rAβ42 oligomers (40 μM) for 1 h (oligomers), rAβ42 oligomers (40 μM) for 1 h and insulin (100 μM) for 20 h (oligomers + insulin). Cells were incubated with Mito Red and Hoechst 33258. The images of each sample were merged. Red staining indicates mitochondrial activity (see red arrows). Blue staining indicates nuclear fragmentation (see blue arrows).

stress stimuli. To test whether the insulin can play its protective role by activating or improving activation of Hsp70, a Western blot experiment was performed. Same amounts of proteins extracted from cells, (i) untreated, (ii) A-betatreated, (iii) treated with insulin alone, and (iv) previously treated with A-beta, were loaded on a SDS-PAGE. The immunoblot was incubated with antibodies against both the constitutive and inducible Hsp70 stress proteins and antiβactin for normalizing gel loading. Levels of the constitutively expressed Hsp70 form (Hsc70) do not change after any stimulus. In agreement with other studies [17], A-beta presence increased the Hsp70 stress-inducible form (Hsp70) as compared with the control (Figure 5). In contrast no activation was detected when LAN5 cells were incubated with insulin alone. Moreover, insulin enhanced A-beta mediated activation of Hsp70 suggesting that this major induction could be necessary to complete a cell survival program.

4. Discussion

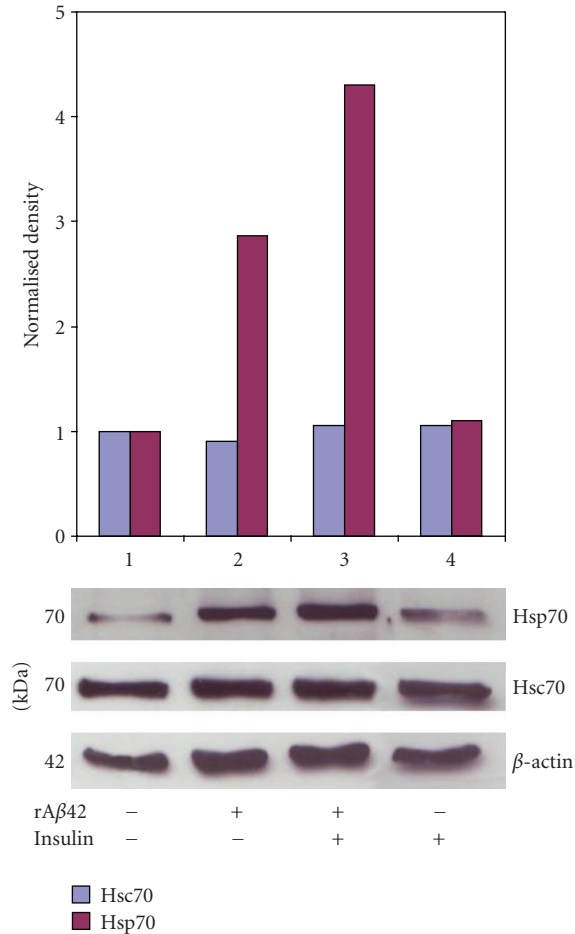An increasing number of reports suggest that A-beta accumulates inside neurons with aging and that A-beta

FIGURE 5: Insulin enhances inducible Hsp70 activation triggered by rAβ42 oligomers. Western blot of proteins extracted from LAN5 untreated (1), treated with rAβ42 oligomers (40 μM) for 1 h (2), rAβ42 oligomers (40 μM) for 1 h and insulin (100 μM) for 20 h (3), insulin alone (100 μM) for 20 h (4), incubated with anticonstitutive Hsp70 (Hsc70), antiinducible Hsp70 (Hsp70) or beta-actin (β-actin). Uniformity of gel loading was confirmed with β-actin as standard. Quantification of immunoreactivity was performed using densitometric analysis.

aggregation can be an initial event for the characteristic cell degeneration present in the Alzheimer's pathology. A-beta aggregated molecules alter normal cell homeostasis including impaired glucose/energy metabolism, mitochondrial dysfunction and oxidative stress [18]. Recently, several studies suggest that disturbance in insulin metabolism, especially insulin resistance, plays a role in the onset and development of AD. Thus, growing evidence begins to find links between AD and type 2 diabetes and, for this reason, AD has been considered the "brain-type diabetes" [5, 19]. Here we provide evidence that insulin can protect cells against damage induced by A-beta oligomers avoiding the apoptosis program.

We found that insulin inhibits A-beta cell death in LAN5 neuroblastoma cells in a dose-dependent manner. According to several results different are the hypothesis reported to explain a mechanism underlying the protective role played by insulin to contrast the impairment produced by A-beta oligomers. Among the first studies, it has been demonstrated that specifically dibutyryl cAMP or insulin inhibit toxic effect of A-beta$_{22-35}$ in cultured rat hippocampal neurons and a recover of the intracellular signal transduction disorder was suggested [11]. More recently, on the basis that A-beta and insulin are both amyloidogenic peptides sharing a common sequence recognition motif [20], it has been reported that A-beta hinders the insulin binding to its receptor (IR), leading to an IR reduced autophosphorylation [12]. The authors suggest that the link between A-beta and insulin is associated to the impairment of glucose utilization, and in agreement to this hypothesis, a recovery of cell viability, through insulin administration could be due to the retrieval of normal glucose metabolism. Other authors [10] suggest that insulin inhibits A-beta fibril network formation, an essential step in exerting its toxicity, at the cell surface of human brain pericytes (HBP). In fact, the HBP line does not express IR, therefore in this case the protective effect of insulin can not be due to its reduced binding to IR. All the results reported above underline that insulin is able to recover an intracellular damage induced by A-beta, whose effect is to produce cellular stress and degeneration. Apoptosis has been often associated to neurodegeneration [21] and to find a mechanism that control caspase activation could be a promising approach to hinder the cell death process. By specific assays, we demonstrate that insulin opposes to apoptotis and particularly caspase 9 and 3 activation after A-beta induced toxicity. According to previous results caspase 8 was not activated by A-beta oligomers, because the extrinsic pathway is a preferential process activated by larger A-beta fibrils [15]. Caspase 9 activation is a consequence of mitochondrial dysfunction often provoked by oxidative stress, and increased oxidative stress has been implicated in the etiology of several pathology including diabetes and AD [22, 23]. Mitochondria are both the major generators and direct targets of reactive oxygen species (ROS) and this had led to the idea that oxidative stress and mitochondrial damage are contributory factors to several disorders. Moreover, it has been demonstrated that, in the presence of A-beta, insulin prevents the decline in mitochondrial oxidative phosphorylation efficiency and avoids an increase in oxidative stress [24]. These data are in agreement with the result here shown that the mitochondrial activity is impaired when the neuroblastoma cells are treated with A-beta oligomers and that this effect is recovered when insulin is added. Moreover, insulin prevents apoptotic pathway activation as observable by the absence of nuclear fragmentation, that instead is well evident in the oligomers treated cells. Thus, we suggest that insulin prevents mitochondrial dysfunction probably by inhibition of ROS formation and activating specific cell signaling (work in progress). Further, it is known that insulin activates the serine-threonine kinase Akt, a protein downstream of PI3K, involved in survival pathway [25–27]. Akt has been well demonstrated to phosphorylating and increasing the expression of a number of proteins involved in apoptotic signaling cascade such as the Bcl-2 family [28, 29]. Presence of A-beta produces a toxic effect, responsible for the observed neuron death. Some

evidences point out the role of molecular chaperones in neurodegenerative processes [30, 31]. We show that a stress response is activated by the increased expression of inducible Hsp70. Interestingly, A-beta is able to activate Hsp70 but, in the present experimental conditions, the endogenous stress response is insufficient to revert the apoptotic pathway. Upregulation of Hsp70 has been found in neurons treated with A-beta and a modulator role in A-beta toxicity has been proposed [17]. The authors hypothesize that an imbalance between the neuronal Hsp protective capacity and the toxic accumulation of A-beta could be the cause of the neuronal death. The presence of insulin enhances the activation of the stress proteins, suggesting that an increased survival program has been activated and the neurons recover their viability. Moreover, it has been demonstrated that Hsp70 is induced by PI3/Akt activation [32] that in turn is activated by insulin. Heat shock proteins can neutralize the neurotoxicity in animal models suggesting potential therapeutic approaches in neurodegeneration associated with abnormal folding and toxicity [33, 34]. Other observations have demonstrated the presence of Hsp70 in AD brain [35]. Hsp70 plays a role in preventing protein aggregation and degradation [36, 37]. Helped by insulin, that increases its expression and activation, we can assume that Hsp70 could prevent A-beta oligomers by successive aggregation steps or promote oligomers degradation.

5. Conclusions

The present data point out the protective action of insulin in inhibiting A-beta cell toxicity and they could be an initial step to understand as insulin resistance or disturbance in insulin metabolism can contribute to neurodegeneration. Moreover, insulin may constitute a therapeutic agent against AD, activating cell survival signaling pathways.

Acknowledgment

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