Research report

Insulin affects synaptosomal GABA and glutamate transport under oxidative stress conditions

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Abstract

In this study, we investigated the in vitro effect of exogenously administered insulin on the susceptibility to oxidative stress and on the accumulation of the amino acid neurotransmitters γ-aminobutyric acid (GABA) and glutamate in a synaptosomal fraction isolated from male Wistar rat brain cortex. Insulin (1 μM) did not affect synaptosomal lipid peroxidation induced by the oxidant pair ascorbate/Fe^{2+}, although under these conditions an increase in thiobarbituric acid reactive substances (TBARS) levels was observed. Under control conditions, the presence of insulin did not change the uptake of [^{3}H]GABA or [^{3}H]glutamate. In contrast, under oxidizing conditions, we observed a 1.8- and a 2.2-fold decrease in [^{3}H]GABA and [^{3}H]glutamate accumulation, respectively, and insulin reverted the lower levels of both [^{3}H]GABA and [^{3}H]glutamate accumulation (to 86.74 ± 6.26 and $67.01\pm6.65\%$ of control, respectively). Insulin also increased the extrasynaptosomal levels of GABA and glutamate, determined both in control and oxidizing conditions. From this study, we can conclude that insulin is a modulator of amino acid neurotransmitter transport, either directly, as seems to occur under normal conditions, or via the decrease in ATP levels and the subsequent reversion of the amino acid transporters, as seems to occur under oxidative stress conditions. The modulation of both GABA and glutamate transport might be implicated in the neuroprotective role of insulin.

1. Introduction

Oxidative stress has been associated with the impairment of cellular function and ultimately with cell death [40], occurring in chronic neurodegenerative and age-related diseases, stroke, and chronic complications of diabetes involving the central nervous system [6,16]. One of the main characteristics of oxidative stress is lipid peroxidation [36], which may induce changes in the structure and function of biological membranes, leading to the alteration of the structural order of membrane lipids [32], membrane permeability to ions [27] and activity of membrane-bound enzymes [34]. The brain is especially

vulnerable to reactive oxygen species (ROS), due to its enrichment in polyunsaturated fatty acids, its high oxygen consumption, its high content in transition metals (such as Fe^{2+}) and poor antioxidant defenses [25].

Although peripherally produced insulin can be transported to the brain via the cerebrospinal fluid, some recent evidence suggests that it can be synthesized de novo by neurons. Schechter et al. [49,50] and Zhao et al. [65] observed the presence of preproinsulin I and II mRNA or insulin receptor (IR) mRNA in cultured neurons and in vivo, using PCR, in situ hybridization and Northern blot techniques, and insulin immunoreactivity in the endoplasmic reticulum and Golgi apparatus, suggesting that it can be synthesized in the brain.

In central nervous system (CNS), insulin is thought to play a complex, but important role, which may involve the regulation of brain metabolism [45,51], neuronal growth

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and differentiation [35,50], or neuromodulation [52,59]. Insulin may also have a neuroprotective effect per se, since it was reported that it reduces cerebral brain damage during stress conditions, like ischemia and traumatic brain injury [4,45]. Brain glucose utilization and metabolism are essential to cognitive functions, and a disturbance in both or the desensitization of insulin receptors, at the brain level, seems to be related to the intellectual decline in Alzheimer's disease and related disorders [19,29]. Excitotoxicity, defined as the excessive stimulation of excitatory amino acid neurotransmitter receptors by endogenous glutamate or aspartate [2], and oxidative stress are two related phenomena that have been shown to be involved in neuronal degeneration occurring in Alzheimer's disease.

The aim of the present work is to evaluate the interactions between exogenously administered insulin and the accumulation of the amino acid neurotransmitters GABA and glutamate in synaptosomes isolated from the cerebral cortex of Wistar rats, under control and oxidative stress conditions. The oxidant pair ascorbate/Fe^{2+} was used to induce oxidative injury and the extent of lipid peroxidation was followed by measuring the formation of thiobarbituric acid reactive substances (TBARS).

2. Material and methods

2.1. Animals

Fifteen male Wistar rats of about 30.88 ± 1.02 weeks of age were obtained from our local colony (Animal Research Center Laboratory, University Hospitals, Coimbra). Animals were kept under controlled light and humidity conditions, and with free access to powdered rodent chow (diet C.R.F. 20; Charles Rivers, France) and water.

2.2. Materials

All chemicals used were of the highest grade of purity commercially available. [³H]GABA (65 Ci/mmol) and L-[G-³H]glutamate (49 Ci/mmol) were from Amersham International (Little Chalfont, UK).

2.3. Preparation of synaptosomes

Crude synaptosomes were prepared from rat brains, according to a pre-established method [15], with some modifications. After animal decapitation, the whole cerebral cortices were rapidly removed and homogenized in 10 volumes of homogenization medium (0.32 M sucrose, 10 mM Hepes and 0.5 mM EGTA-K^+, buffered with Tris at pH 7.4). The homogenate was centrifuged at $1000 \times g$ for 5 min, the synaptosomal fraction was isolated from the supernatant by centrifugation at $12\,000 \times g$ for 10 min and centrifuged again at $12\,000 \times g$ for 10 min in 10 ml of washing medium (0.32 M sucrose, 10 mM Hepes, buffered

at pH 7.4 with Tris). The white and fluffy synaptosome layer without contaminant mitochondria (mitochondria free), was then resuspended, respun and resuspended in the sucrose medium at a protein concentration of 15–20 mg/ml, as determined by the biuret method [24]. Experiments were carried out within 3 h after synaptosomal fraction preparation.

2.4. Induction of oxidative stress

The oxidizing agents, ascorbic acid and iron (Fe^{2+}, ferrous sulfate) were used to induce oxidative stress [64]. Synaptosomes (1 mg/ml) were peroxidized by incubation for 15 min at 30 °C, in a standard medium containing (in mM): 132 NaCl, 3 KCl, 1 $MgCl_2$, 1 NaH_2PO_4, 1.2 $CaCl_2$, 10 glucose and 20 mM Hepes adjusted to pH 7.4 with Tris, supplemented with 0.8 mM ascorbic acid and 2.5 µM iron. Ascorbic acid and iron solutions were prepared immediately before use and protected from light. Similar experiments were carried out in the presence of 1 µM insulin, with synaptosomes preincubated for 15 min before the addition of ascorbate/Fe^{2+}.

2.5. Evaluation of lipid peroxidation

The extent of lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS), using the thiobarbituric acid assay (TBA) [12]. The amount of TBARS formed was calculated using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and expressed as nmol TBARS/mg protein.

2.6. Determination of [³H]GABA and [³H]glutamate uptake

The uptake of [³H]GABA and [³H]glutamate was determined as previously described [44,60]. Synaptosomes at a concentration of 0.5 mg of protein/ml were equilibrated at 30 °C in a Na^+ medium containing (in mM): 125 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 $CaCl_2$, 3 glucose and 10 Hepes–Tris at pH 7.4 in the absence or presence of ascorbic acid (0.8 mM) plus iron (2.5 µM). The same experimental procedure was carried out in the presence or absence of insulin 1 µM, with synaptosomes preincubated for 15 min before the addition of ascorbate/Fe^{2+}. Aminooxyacetic acid (10 µM) was included in all media when studying the uptake of [³H]GABA to prevent GABA metabolism. After 15 min of incubation, the uptake reaction was started by adding [³H]GABA (final concentration 0.5 µM, 0.25 µCi/ml) or [³H]glutamate (final concentration 10 µM, 49 Ci/mmol). Uptake was stopped at 10 min by rapid filtration of the samples (0.2 ml) under vacuum, through glass fiber filters Whatman GF/B, fol-

lowed by a wash with 10 ml of the Na^+-containing-glucose-free medium (washing medium). The filters were placed in scintillation vials with 3 ml of scintillation Universol cocktail (ICN, Irvine, CA, USA) and radioactivity was counted in a liquid scintillation analyzer Packard Tri-Carb 2500 TR. The quenching of radioactivity in the samples was corrected automatically using an efficiency correlation curve obtained for ^3H-quenched standards by the external standardization method. The results were presented as percentage of the control, considering the mean of controls as 100%.

2.7. Flurimetric determination of extrasynaptosomal levels of amino acids following HPLC separation

The extrasynaptosomal levels of the amino acids GABA and glutamate were analysed fluorimetrically following o-phthaldialdehyde (OPA)/2-mercaptoethanol derivatization and HPLC separation, as described by Sitges et al. [53], with some modifications. The amino acids were separated by reverse phase Gilson-ASTED HPLC system, composed of a Spherisorb ODS column (particle size, 5 μm; 150 mm long; 4.6 mm i.d.) at 25 °C, and a Gilson, model 121, fluorescence detector set, at 340 nm (excitation wavelength) and at 410 nm (emission wavelength). A linear gradient elution program carried out over 30 min was applied for amino acid elution: eluent A (30 mM sodium acetate buffer, pH 6.8) from 100 to 50%, and eluent B (methanol) from 0 to 50%, with a flow rate of 2.5 ml/min. Amino acids were detected as fluorescence derivatives after precolumn derivatization with OPA/MCE (orthophthaldialdehyde/2-mercaptoethanol). The integration of the amino acid peak area and further calculations were carried out by Gilson system software, and quantification was allowed by running standard amino acids solutions under the same conditions.

2.8. Synaptosomal membrane potential measurements

The synaptosomal transmembrane potential ($\Delta\Psi$) was monitored by evaluating the transmembrane distribution of TPP^+ (tetraphenylphosphonium) with a TPP^+-sensitive electrode prepared according to Kamo et al. [21], using a calomel electrode as reference. Reactions were carried out in a chamber with magnetic stirring in 1 ml of Na^+ medium containing (in mM): 125 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 $CaCl_2$, 3 glucose and 10 Hepes–Tris at pH 7.4. Synaptosomes (0.5 mg/ml) were equilibrated in Na^+-medium for 15 min at 30 °C in the absence or presence of insulin (1 μM) and then incubated for the same time with or without ascorbate/iron. The experiments were started by adding 3 μM TPP^+ and the membrane potential was estimated from the decrease in TPP^+ concentration in the reaction medium.

2.9. Data analysis and statistics

Results are presented as mean±S.E.M. of the indicated number of experiments. Statistical significance was determined using the Student's t-test or the one-way ANOVA test for multiple comparisons. A P-value <0.05 was considered significant.

3. Results

3.1. Synaptosomal susceptibility to oxidative stress induced by ascorbate/iron

In the present study, oxidative stress was induced with 0.8 mM ascorbate and 2.5 μM Fe^{2+}. Under these conditions, an increase in lipid peroxidation of synaptosomes, as determined by the formation of TBARS, has been reported [10,46]. Fig. 1 shows that, under control conditions, the synaptosomal levels of TBARS were similar in the absence and in the presence of insulin (1 μM) (2.59 ± 0.32 and 2.28 ± 0.27 nmol/mg protein, respectively). The incubation of synaptosomes with ascorbate/Fe^{2+} for 15 min (oxidized synaptosomes) resulted in a significant increase in the production of TBARS to 23.42 ± 2.28 nmol/mg protein. Under these conditions, the presence of insulin did not significantly change the synaptosomal levels of TBARS (20.57 ± 2.33 nmol/mg protein).
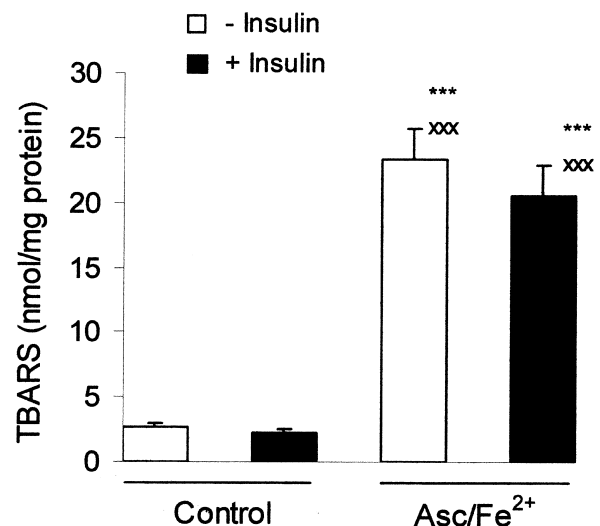

Fig. 1. Effect of oxidative stress induced by ascorbate/iron on the extent of lipid peroxidation on synaptosomes isolated from rat brain. Synaptosomes were preincubated in Na^+ medium in the absence or presence of insulin 1 μM for 15 min at 30 °C, and then incubated in the presence (Asc/Fe^{2+}) or in the absence (control) of ascorbate (0.8 mM) and iron (2.5 μM) for 15 min. The extent of lipid peroxidation was evaluated by determining the production of TBARS and expressed as nmol/mg protein. Data are the means±S.E.M. of five to seven independent experiments. Statistical significance: ***$P<0.001$ compared to the control not treated with ascorbate/iron; $^{\times\times\times}P<0.001$ compared to the control treated with 1 μM insulin in the absence of ascorbate/iron.
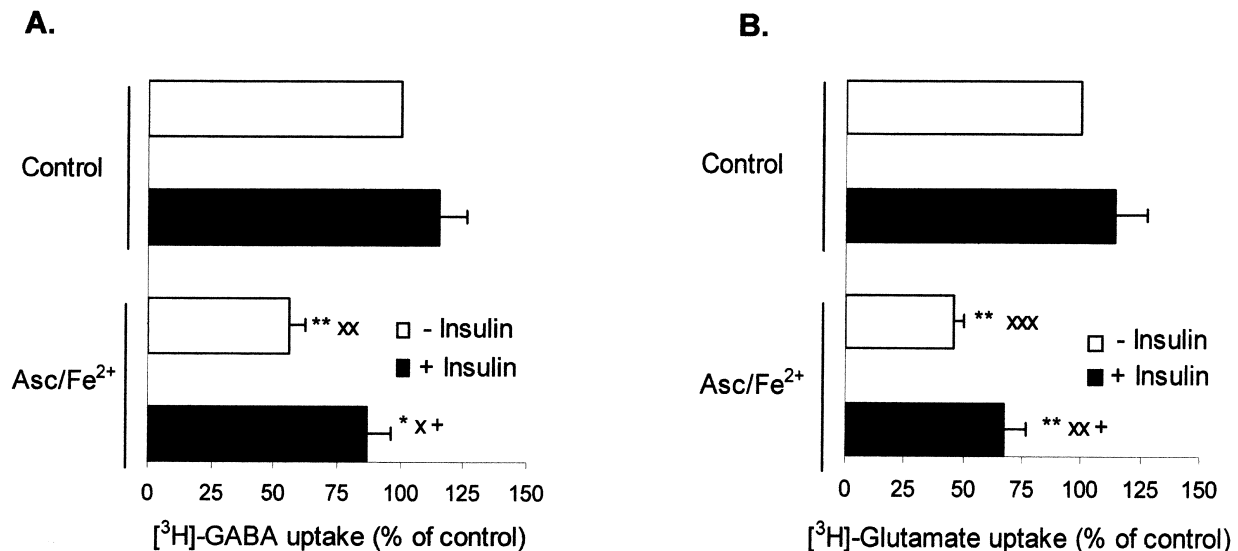
A.

B.

Fig. 2. Effect of insulin on the uptake of [³H]GABA (A) and [³H]glutamate (B) by synaptosomes submitted (Asc/Fe²⁺) or not (control) to oxidative stress induced by ascorbate/iron. Synaptosomes were equilibrated in Na⁺-medium for 15 min at 30 °C in the absence or presence of insulin (1 μM), and then incubated with or without ascorbate/iron for 15 min. The [³H]GABA or [³H]glutamate uptake reaction was started by the addition of [³H]GABA or [³H]glutamate and was carried out for 10 min. Data, expressed as a percentage of [³H]GABA uptake or [³H]glutamate uptake in control synaptosomes (considering 118.69 pmol [³H]GABA/mg protein and 0.79 nmol [³H]glutamate/mg protein as 100%), are the means±S.E.M. of four to five and of four to seven different experiments, respectively. Statistical significance: *$P<0.05$; **$P<0.01$ compared to the control not treated with ascorbate/iron; $^{\times}P<0.05$; $^{\times\times}P<0.01$; $^{\times\times\times}P<0.001$ compared to the control treated with 1 μM insulin in the absence of ascorbate/iron; $^{+}P<0.05$ compared to the control treated with ascorbate/iron.

3.2. Effect of insulin on the uptake of [³H]GABA and [³H]glutamate under oxidative stress conditions

Fig. 2A shows that the synaptosomal uptake of [³H]GABA, under control conditions, was not significantly altered in the presence of 1 μM insulin (100.0±0.0 and 114.97±11.28% of control, in the absence and presence of insulin, respectively). Ascorbate/Fe²⁺ treatment decreased significantly the synaptosomal accumulation of [³H]GABA (to 56.14±8.06% of control), which was significantly increased in the presence of insulin (to 86.74±6.26% of control). In Fig. 2B, we can observe that the synaptosomal accumulation of [³H]glutamate in the presence of insulin was not significantly affected (113.96±10.53% of control). After the incubation of synaptosomes with ascorbate/Fe²⁺, a significant decrease in the accumulation of [³H]glutamate was observed (to 46.01±3.19% of control). Under oxidative conditions, this uptake value was significantly increased by the incubation of the synaptosomal preparation with insulin (to 67.01±6.65% of control). The amounts of [³H]GABA and [³H]glutamate accumulated in control synaptosomes were 118.69 pmol/mg protein and 0.79 nmol/mg protein, respectively.

3.3. Effect of oxidative stress and/or insulin on the extrasynaptosomal levels of GABA and glutamate

Under control conditions, the amount of extrasynap-

tosomal GABA was 9.18±0.59 nmol GABA/mg protein and the synaptosomal incubation with insulin 1 μM significantly increased the extracellular content of this amino acid to 12.43±1.80 nmol GABA/mg protein (Fig. 3A). Under oxidizing conditions, the extrasynaptosomal levels of GABA remained unaffected (10.40±0.76 nmol/mg protein), whereas insulin induced a significant increase in the content of this amino acid (to 14.68±1.62 nmol/mg protein).

Under control conditions, extrasynaptosomal levels of glutamate were about 17.76±0.4 nmol/mg protein. In the presence of insulin, a significantly higher extracellular amount of glutamate could be determined (27.04±1.30 nmol/mg protein). Oxidizing conditions induced an increase in the extrasynaptosomal content of glutamate, to a value of 22.01±1.46 nmol/mg protein, and incubation with insulin under such conditions further increased those values (to 31.83±1.17 nmol/mg protein) (Fig. 3B).

3.4. Effect of oxidative stress and/or insulin on synaptosomal transmembrane potential (ΔΨ)

Fig. 4 shows that the synaptosomal transmembrane potential (ΔΨ), under control conditions, is not altered in the presence of 1 μM insulin (−96.72±6.06 and −99.41±8.99 mV, in the absence and presence of insulin, respectively). Ascorbate/Fe²⁺ treatment significantly decreased ΔΨ values (to −83.20±7.75 mV), which were not affected in the presence of insulin (−84.52±6.55 mV).
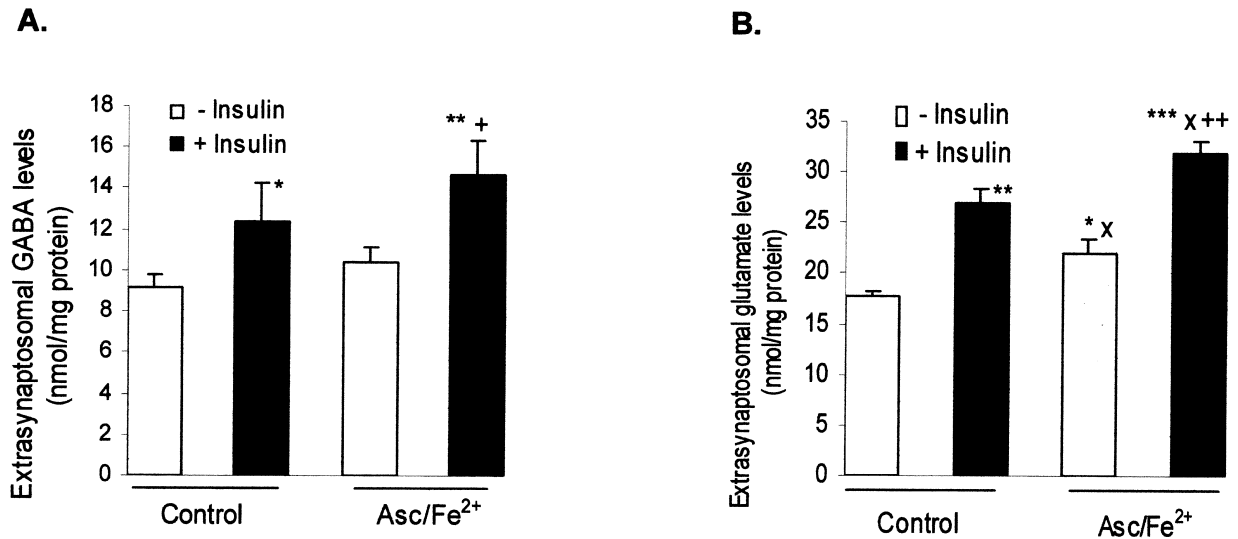
A.

B.

Fig. 3. Effect of insulin on the release of the endogenous amino acids GABA (A) and glutamate (B) by synaptosomes submitted (Asc/Fe^{2+}) or not (control) to oxidative stress induced by ascorbate/iron. Synaptosomes were exposed to ascorbate (0.8 mM) and iron (2.5 μM) for 15 min at 30 °C. Control synaptosomes were preincubated for 15 min in the absence of the oxidizing agents, but in the absence or in the presence of insulin 1 μM. Amino acids were analyzed fluorimetrically by HPLC with o-phthaldialdehyde (OPA)/2-mercaptoethanol derivatization. The values, expressed in nmol/mg protein, are the mean±S.E.M. of three to five independent experiments. Statistical significance: *P<0.05; **P<0.01; ***P<0.001 compared to the respective control not treated with ascorbate/iron; $^{×}$P<0.05 compared to the control incubated in the presence of insulin; $^{+}$P<0.05; $^{++}$P<0.01 compared to the control in the presence of ascorbate/iron.

Fig. 4. Effect of insulin on the transmembrane potential (ΔΨ) in synaptosomes submitted (Asc/Fe^{2+}) or not (control) to oxidative stress induced by ascorbate/iron. Synaptosomes (0.5 mg/ml) were equilibrated in 1 ml of Na^{+}-medium for 15 min at 30 °C in the absence or presence of insulin (1 μM) and then incubated for 15 min with or without ascorbate/iron. The experiments were started by adding 3 μM TPP^{+} and the membrane potential was estimated from the decrease in TPP^{+} concentration in the reaction medium. Data, expressed as –mV, are the means±S.E.M. of nine to thirteen 13 different experiments. Statistical significance: **P<0.01 compared to the control not treated with ascorbate/iron; $^{××}$P<0.01 compared to the control treated with 1 μM insulin in the absence of ascorbate/iron.

4. Discussion

In the present study, we evaluated the effects of insulin on the uptake of the amino acid neurotransmitters, GABA and glutamate, by synaptosomes submitted to oxidative stress conditions. The ascorbate/Fe^{2+}-induced increase in TBARS was not reverted by insulin, suggesting that other mechanisms, rather than a direct action of insulin on membrane lipid peroxidation may mediate its possible neuroprotective role. Although there are no published data on iron chelation by insulin, we cannot rule out an eventual chelation effect of iron by insulin, similar to that of zinc [11,22], as a potential protective role of insulin against oxidative stress. Despite the criticisms to the TBARS assay [20,63], it was recently demonstrated that it may provide a simple, rapid and sensitive method to evaluate the extent of lipid peroxidation in brain preparations [48], showing well-correlated results with the determination of conjugated dienes, polyunsaturated fatty acid or carbonyl groups [37].

Anomalous synaptic signaling has been implicated in some neurodegenerative disorders, such as Alzheimer's and Huntington's diseases, stroke and epilepsy [5,8]. Oxidative stress seems to be involved in neuronal cell death [54] and in synaptic degeneration [28] associated with neurodegenerative diseases. Membrane lipid peroxidation seems to play a major role in the increased synaptic vulnerability to excitotoxicity, since it impairs the function of membrane ATPases, and of glucose and glutamate transporters [8,13].

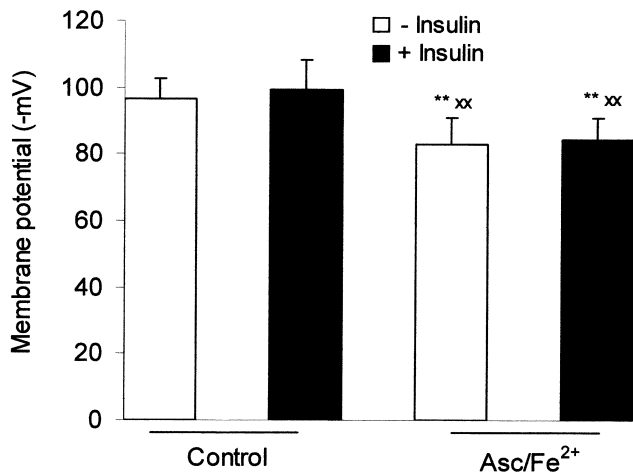The decrease in synaptosomal GABA and glutamate

accumulation, observed under ascorbate/Fe^{2+}-induced oxidizing conditions, is consistent with a previous study carried out in our laboratory [10], suggesting an excitotoxic role for oxidative stress, since the main functional role of glutamate transporters, especially in glial cells, is to maintain low glutamate concentration in the extraneuronal space [30,42].

The oxidative stress-induced decrease in synaptosomal GABA uptake might result from a partial inhibition of the GABA carrier, caused by a change in membrane order or by the dissipation of the Na^+ gradient after ascorbate/Fe^{2+}-induced lipid peroxidation [33]. This decrease in inhibitory neurotransmission might promote neuronal damage in oxidative stress-related diseases and aging [43]. Despite our observation of decreased GABA accumulation, we did not report significant changes in extrasynaptosomal GABA levels. However, other authors described an oxidative stress-induced release of the inhibitory amino acids GABA, glycine and taurine, suggesting a protective mechanism against oxidative stress-related neuronal death [26,40]. Our observation of decreased glutamate accumulation and increased extrasynaptosomal glutamate levels under ascorbate/Fe^{2+}-induced oxidation further support this hypothesis, since oxidative stress-induced damage to nerve cell membrane, leads to the release and extracellular accumulation of the excitatory amino acids, aspartate and glutamate, contributing to excitotoxicity [47], which further promotes ROS generation [3].

The ROS-induced decrease in glutamate uptake may result from a decrement in both glutamate uptake affinity and velocity [42], as a result of the oxidation of glutamate transporter sulphydryl groups [1,58], membrane lipid peroxidation-induced changes in the transporters function, or the impairment of Na^+,K^+ ATPase activity [17]. The progressive weakening of ionic gradients with the concomitant impairment on the uptake of glutamate and the subsequent reversion of the glutamate transporter, leads to the release of toxic amounts of this neurotransmitter [58]. Our study also showed an oxidative stress-induced decrease in synaptosomal plasma membrane potential, despite an increase in ATP levels, suggesting that the oxidative stress-induced impairment of Na^+,K^+ ATPase activity could contribute to the intrasynaptosomal increase in ATP and to the observed membrane depolarization, accompanied by an increment in intracellular Na^+ and Ca^{2+} levels, thus favouring the Ca^{2+}-induced release of neurotransmitters [57].

Insulin-induced rapid translocation of $GABA_A$ receptors to the postsynaptic domain might enhance inhibitory synaptic transmission after an increase in GABA release, thus supporting the idea of a neuroprotective role of insulin [62]. However, some controversy exists on the modulatory effect of insulin on amino acid accumulation in brain [7,41,52]. Insulin stimulated rat brain synaptosomal uptake of amino acid neurotransmitters with a dose-dependent effect [41], with 3 μM increasing GABA and glutamate

accumulation [18,41]. Insulin could also participate in GABA clearance through the regulation of its synthesis and transporter activity.

Rhoads et al. [41] demonstrated that 1 μM was the lowest insulin concentration that increased glutamate uptake by cortical synaptosomes, on an effect maintained from 1 to 20 min of in vitro incubation. In this study, we used an in vitro insulin concentration of 1 μM, incubated for 15 min with the synaptosomal fraction, in the absence or presence of the oxidizing pair ascorbate/Fe^{2+}. Our observation that insulin 1 μM did not affect per se the uptake of GABA is consistent with the observations of Lajtha and Sershen [23]. The increase in both GABA and glutamate extrasynaptosomal levels might be due to the capacity of insulin to stimulate protein synthesis and expression [31,39] or, in the case of GABA, to the stimulation of ornithine decarboxylase, responsible for the conversion of ornithine to putrescine, a precursor of GABA, thus increasing its synthesis [51]. Insulin's hypothetical neuroprotective role against oxidative stress-mediated glutamate neurotoxicity may be exerted directly on membrane transporters, or indirectly through the increase in Na^+,K^+ ATPase activity or in the number of its α1 subunits [56], thus decreasing the intracellular Na^+ and Ca^{2+} levels [41,51] and the vesicle-dependent release of the neurotransmitter (exocytosis). We also observed that insulin apparently did not revert the decrease in synaptosomal membrane potential induced by ascorbate/Fe^{2+} (Fig. 4). These data suggest that the increased extrasynaptosomal levels of GABA and glutamate could result from the decrease in ATP levels and the subsequent inhibition of synaptosomal membrane Na^+,K^+ ATPase activity, thus reverting GABA and glutamate transporters. Although an insulin-induced decrease in ATP levels occurred, it was not sufficient to induce changes in synaptosomal membrane potential under oxidative stress conditions, suggesting that the exocytotic neurotransmitter release might not be involved.

In the presence of ascorbate/Fe^{2+}, the insulin-induced increase in both GABA and glutamate uptake and extrasynaptosomal levels, suggest that insulin may exert a modulatory role in amino acid neurotransmitter transport system. The increase in the uptake of these neurotransmitters may constitute a protective mechanism against oxidative stress-induced increase in extrasynaptosomal glutamate levels. Furthermore, the increase in extrasynaptosomal GABA levels that we observed, also supports this hypothesis. Guyot et al. [14] hypothesized that insulin could stimulate glycolysis and pyruvate dehydrogenase activity, leading to the increment in ATP and lactate, allowing the ion pumps to function properly and thus contributing to the synaptosomal amino acid transport. However, this seems not to be the case in this study, since we did not observe insulin-induced changes in ATP levels under control conditions. Another hypothesis could be the insulin-induced activation of insulin receptor kinase activi-

ty, and the subsequent tyrosine phosphorylation of substrates, like the insulin receptor substrate-1, which may activate the uptake of amino acids [55].

The different results obtained between the present work with normal Wistar rats (insulin-induced increase in both GABA and glutamate transport) and those obtained in a previous study with type 2 diabetic GK rats (an ascorbate/Fe^{2+}-induced decrease in GABA uptake), might be explained by the defects in insulin action in GK rats, since these animals are characterized by insulin resistance, fasting hyperinsulinemia and inhibition of glucose-induced insulin secretion [38,61].

From these results, we can hypothesize that the modulatory effect of insulin could be one of the mechanisms responsible for its neuroprotective role in neurodegenerative disorders, namely in Alzheimer's disease, in which oxidative stress synaptic dysfunction has been shown to occur [9]. Recently, it has been suggested that a dysfunction on the neuronal insulin receptor signal transduction cascade might be central to the ethiopathogenesis of Alzheimer's disease [19], further supporting the hypothesis of a neuroprotective role of insulin under stress conditions.

Acknowledgements

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