The Effect of Metformin and Taurine, Alone and in Combination, on the Oxidative Stress Caused by Diabetes in the Rat Brain

George J. Clark, Kashyap Pandya, and Cesar A. Lau-Cam

Abstract This study has compared the effects of metformin (MET) and taurine (TAU), singly and in combination, on the oxidative stress caused by diabetes in the rat brain. For this purpose, male Sprague-Dawley rats, 200–225 g in weight, assigned to groups of 6, were intraperitoneally (i.p.) treated with the diabetogen streptozotocin (STZ, 60 mg/kg, in citrate buffer pH 4.5) on day 1 and, after 14 days, orally (p.o.) with either MET, TAU or MET-TAU (each at 2.4 mM/kg, in water). Control rats received only citrate buffer pH 4.5 (2 mL) or only STZ on day 1 by the i.p. route. All the animals were sacrificed by decapitation on day 57 and their brains collected by the freeze clamp technique. Blood samples were placed in heparinized tubes and used for the assay of the plasma glucose (GLC) and blood insulin (INS) levels. Immediately thereafter, the brains were surgically removed and a portion was used to prepare a homogenate in 0.1 M PBS pH 7.4, which was used for the assay of indices of oxidative stress. Diabetes raised the plasma GLC level (+313%) but lowered that of the blood INS (−76%) compared to corresponding values from nondiabetic rats. In addition it raised the brain malondialdehyde level (+59%) but lowered the reduced/disulfide glutathione ratio (−46%), and activities of catalase (−43%), glutathione peroxidase (−48%), superoxide dismutase (−65%), glutathione reductase (−50%) and glutathione S-transferase (−51%) significantly (all at $p < 0.001$). Except for the greater decrease in GLC (+90% vs. +22%) and increase in INS (−26% vs. −52%) levels seen in rats receiving MET than in rats receiving TAU, these compounds protected the brain against oxidative stress to significant ($p \leq 0.05\%$) and rather similar extents. Furthermore, the concurrent administration of MET and TAU to the diabetic rats led to brain values of indices of oxidative stress that were lower than those attained with MET alone, although generally not to a statistically significant degree.

Keywords Diabetes • Brain • Oxidative stress • Metformin • Taurine

G.J. Clark • K. Pandya • C.A. Lau-Cam (✉)
Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences,
St. John's University, Jamaica, NY 11439, USA
e-mail: claucam@usa.net

Abbreviations

CAT   Catalase
GLC   Glucose
GPx   Glutathione peroxidase
GR   Glutathione reductase
GSH   Reduced glutathione
GSSG   Glutathione disulfide
GST   Glutathione S-transferase
INS   Insulin
MDA   Malondialdehyde
MET   Metformin
SOD   Superoxide dismutase
TAU   Taurine

1 Introduction

Oxidative stress is known to be at the center of the structural, neurophysiological and neuropsychological alterations associated with long-term effects of diabetes in the brain (Muriach et al. 2014). A common theory to account for the cerebral dysfunction of diabetes is a reduction of antioxidant defenses and a parallel increase in the production of free radical caused by the ensuing hyperglycemia, effects that may leave the brain vulnerable to the damaging effects of free radicals and, hence, to cellular damage and functional impairment (Baquer et al. 1990; Maritim et al. 2003).

   When compared to other organs in the body, the brain is found to be particularly susceptible to the damaging effects of oxidative stress as a result of a high oxygen demand, a high rate of oxidative metabolism, an abundance of redox-active metals such as iron and copper, high levels of peroxidizable polyunsaturated fatty acids, and a relative paucity of antioxidant defenses (Wang et al. 2014; Wang and Michaelis 2010). As a result, an imbalance can develop between oxidative insults such as reactive oxygen species (ROS) and antioxidant defenses, especially antioxidant enzymes, a situation that, in turn, can favor the development of oxidative stress (Bonnefont-Rousselot 2002; Maritim et al. 2003). Specifically, in addition to peroxidative damage of brain lipids, proteins and nucleic acids (Vincent et al. 2004), there is a decrease in the activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and total antioxidant status, and an increase in glucose autoxidation, lipid peroxidation (LPO), nitrosative stress and nitric oxide (NO) formation (Acar et al. 2012; Mastrocola et al. 2005; Muriach et al. 2014). Moreover, the levels of natural antioxidants such as reduced glutathione (GSH) may be diminished as a result of an increase activity of the NADPH-requiring polyol pathway by a high glucose flux, a situation that depletes the availability of NADPH

needed for the enzymatic regeneration of GSH from its oxidized disulfide (GSSG) form (Giacco and Brownlee 2010).

From the results obtained from the brain of rats treated with the diabetogen streptozotocin (STZ) it has become evident that there is an increase in the values of malondialdehyde (MDA), ROS and NO, extent of LPO, and activity of glutathione reductase (GR), changes that are accompanied by a lower activity of brain catalase (CAT), glutathione peroxidase (GPx) and manganese SOD, an impairment of mitochondrial function, membrane potential and production of adenosine triphosphate, and a reduced ratio of GSH/GSSG relative to corresponding results from nondiabetic rats (Acar et al. 2012; Cardoso et al. 2013; Kumar and Menon 1993; Ortiz-Avila et al. 2015).

Previous studies from this laboratory have found that taurine (TAU), an endogenous and conditional β-amino acid, can protect against biochemical, functional and morphological changes caused by diabetes in the plasma, erythrocyte (Budhram et al. 2013) and kidney (Pandya et al. 2013) of rats previously treated with STZ; and that these benefits are also exerted against the oxidative stress that develops in the brain (Patel et al. 2016). Taking into account these results, the present study was specifically aimed at further investigating whether adding TAU to a treatment of diabetes with metformin (MET), a biguanide compound currently used as a first-line oral hypoglycemic for the treatment of diabetes, can lead to a greater protection of the brain than either compound alone against diabetes-related oxidative stress. To this effect, rats previously made diabetic with STZ were separately treated with MET, TAU and MET-STZ for 6 weeks and, in each case, indices of oxidative stress were measured in the brain.

2   Methods

2.1   Animals

Male Sprague-Dawley rats, 200–225 g in weight, acclimated for 2 weeks in a room maintained at a constant humidity and temperature (23 ± 1 °C) and a normal 12 h light—12 h dark cycle. The rats had free access to a commercial rodent diet (LabDiet® 5001, PMI Nutrition International, Brentwood, MO) and filtered tap water during the entire study.

2.2   Treatments and Samples

All the experimental groups consisted of 6 rats. Diabetes was induced with a single 60 mg/kg intraperitoneal (i.p.) dose of STZ in citrate buffer pH 4.5. Starting on day 15 and continuing for the next 41 days, separate groups of diabetic rats received a 2.4 mM/kg, daily dose of either MET, TAU or MET plus TAU in distilled water by

oral gavage. Nondiabetic (control) rats received only citrate buffer pH 4.5 (2 mL, i.p.) on day 1, and physiological saline (2 mL) by oral gavage from day 15 onwards. A diabetic group received no other treatment than one with STZ. All treatments were conducted for a total of 42 days. The animals were sacrificed by decapitation on day 56 and their blood samples collected without delay. A portion of blood sample was mixed with sodium heparin and processed for the plasma fraction The skulls were cut open with the help of a Friedman rongeur to expose the brains, which were immediately removed by the freeze-clamping technique of Wollenberger et al. (1960), immersed in liquid nitrogen, and stored at −80 °C until needed.

2.3   Plasma Glucose (GLC)

The GLC content was measured using a commercial colorimetric kit (Procedure No. 510 from Sigma-Aldrich, St. Louis, MO, USA). The results were expressed in mg/dL.

2.4   Blood Insulin (INS)

The concentration of INS was measured by means of a commercial assay kit (Insulin ELISA kit, Calbiotech Inc., Spring Valley, CA, USA). The results were expressed in μIU/mL.

2.5   Brain MDA

The concentration of MDA was measured as thiobarbituric acid reactive substances (TBARS) by the end point assay method of Buege and Aust (1978). The results were expressed in nM/g of tissue.

2.6   Brain GSH and GSSG

The brain levels of GSH and GSSG were measured fluorometrically by the method of Hissin and Hilf (1976), which is based on the reaction of GSH with *ortho*-phthalaldehyde (OPT) at pH 8.0 and of GSSG with OPT at pH 12.0. Prior to the measurement of GSSG, any interfering GSH was complexed with N-ethylmaleimide according to the method of Güntherberg and Rost (1966) to prevent its interfering effect on the measurement of GSSG. The concentrations of GSH and GSSG were expressed as μM/g of tissue.

2.7   Brain CAT, GPx and SOD

The CAT activity was measured according to Aebi (1984), which is based on the ability of this enzyme to degrade hydrogen peroxide (H_2O_2) to water and oxygen. The result was expressed as U/mg of protein.

The GPx activity was measured by the method of Günzler and Flohé (1985), which is based on the conversion of GSH to GSSH in the presence of an excess of NADPH and GR. The result was expressed as U/mg of protein.

The SOD activity was measured using the method of Misra (1985), which is based on the inhibitory action of this enzyme on the autoxidation of epinephrine by the superoxide radical to a pink adrenochrome. The result was expressed as U/mg of protein.

2.8   Brain GR and GST

The GR activity was measured as described by Delides et al. (1976), based on the ability of this enzyme to convert GSSG to GSH in the presence of NADPH. The decrease in absorbance at 340 nm reflected the formation of $NADP^+$ from NADPH. The GR activity was calculated using an extinction coefficient of $6.22\ mM^{-1}$, and was reported as μM of NADPH consumed/mg of protein.

The GST activity was measured according to the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The rate of formation of CDNB-GSH conjugate, reflecting the GST activity, was followed for 5 min at 340 nm. The GST activity was calculated using an extinction coefficient of $9.6\ mM^{-1}$, and was reported as μM of CDNB-GSH conjugate formed/min/mg of protein.

2.9   Brain Protein

The protein content of the brain sample was measured by the Lowry method (Lowry et al. 1951), using a bovine serum albumin calibration curve covering the concentration range $1.95 \times 10^{-3} - 2 \times 10^3$ μM. The results were expressed in μg/mL of sample.

2.10   Statistical Analysis of Data

Experimental results are presented as mean ± SEM for n = 6. Statistical comparisons were against both normal rats and untreated diabetic rats, and were made by unpaired Student's t-test with the help of a commercial software system (JMP® 7, JMP® Statistical Discovery Software, Cary, NC, USA). Differences were considered to be statistically significant at $p \leq 0.05$.

3 Results

3.1 Effects on Circulating GLC and INS Levels

The development of type 2 diabetes was ascertained from the circulating levels of both GLC and INS shown in Table 1. Diabetic animals showed much higher levels of plasma GLC than nondiabetic ones by the end of 8 weeks (+313%, $p < 0.001$). A daily treatment of the diabetic rats with MET reduced this increase markedly (+90%, $p < 0.001$), an effect that was ~2.5-fold greater than one with TAU (+222%, $p < 0.001$). Treating the diabetic rats with MET plus TAU led only to a small increase in potency relative to MET alone (+84%, $p < 0.001$). In terms of the plasma INS, it was determined that at the end of 8 weeks diabetes had reduced the level significantly (−76%, $p < 0.001$ vs. control). This effect was significantly antagonized by both MET and TAU, with the former compound appearing twice as potent (−26%, $p < 0.01$) than the latter (−52%, $p < 0.001$). On the other hand, a treatment with MET-TAU further reduced the inhibitory action of diabetes on INS secretion (−18%, $p < 0.05$) although the effect was not significantly different from that attained with MET alone. Neither TAU or MET were found to significantly affect the basal circulating levels of both GLC and INS.

3.2 Effects on Brain MDA, GSH and GSSG Levels, and GSH/ SSG Ratio

As shown in Fig. 1, the brain of diabetic rats contained higher levels of MDA (+59%, $p < 0.001$) than the brain from normal rats. This increase was reduced by about one-half by a daily treatment with either MET (+29%, $p < 0.01$) or TAU (+28%, $p < 0.01$); and still further when the two compounds were given concurrently (−10%). Neither MET nor TAU were found to raise the basal levels of MDA to a significant extent (≤5%).

Table 1 The effects of MET, TAU and MET-TAU on the plasma GLC and blood INS levels of rats made diabetic with STZ[a,b]

Group	Plasma GLC, mg/dL	Blood INS, µIU/mL
Control	103.59 ± 5.06[+++]	44.08 ± 2.46[+++]
STZ	428.08 ± 21.77[***]	10.58 ± 1.33[***]
MET	101.54 ± 5.57[+++]	44.31 ± 1.71[+++]
TAU	102.05 ± 3.33[+++]	42.94 ± 2.37[+++]
MET-STZ	196.41 ± 5.00[***,+++]	32.50 ± 2.35[**,+++]
TAU-STZ	333.37 ± 6.60[***,++]	20.94 ± 1.83[***,+++]
MET-TAU-STZ	190.43 ± 2.22[***,+++]	36.02 ± 1.87[*,+++]

[a]Values are shown as the mean ± SEM for n = 6
[b]Statistical comparisons were significantly different from Control at [*]$p < 0.05$, [**]$p < 0.01$ and [***]$p < 0.001$; and from STZ at [++]$p < 0.01$ and [+++]$p < 0.001$
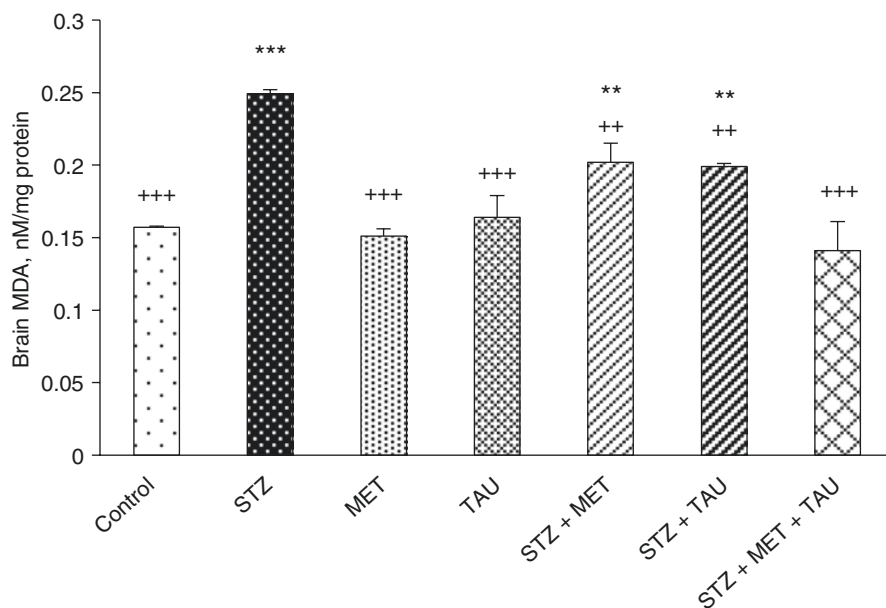
Fig. 1 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain MDA levels of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at **p < 0.01 and ***p < 0.001; and from STZ at $^{++}$p < 0.01 and $^{+++}$p < 0.001

From the results presented in Fig. 2, it is apparent that diabetes lowered the brain levels of GSH (−79%, p < 0.001) and GSSG (−62%, p < 0.001) to a significant extent (p < 0.001 with both vs. control values). Both MET (−25%, p < 0.01) and TAU (−28%, p < 0.01) were able to reduce the loss of GSH to about the same extent, an effect that was somewhat enhanced (−18%, p < 0.05) when these compounds were administered alongside. A similar trend of effects was observed in terms of the reduction of the GSSG levels by diabetes, with MET (−22%, p < 0.01) appearing slightly less effective than TAU (−18%, p < 0.05), and about equipotent when provided together with TAU (−25%, p < 0.01). On the other hand, neither compound affected the basal levels of GSSG. From these results, it was determined that diabetes lowered the GSH/GSSG ratio by 46% (p < 0.001 vs. control), that both MET and TAU were able to nullify this effect, and that a combined treatment with these compounds was able to reverse the effect (+9%) (Fig. 3).

3.3 Effects on Brain GR and GST Activities

The brain activity of GR was markedly decreased in diabetic rats (−50%, p < 0.001) relative to that in normal rats (Fig. 4). This effect was significantly attenuated by either MET (−27%, p < 0.01) or TAU (−29%, p < 0.01) and also by their
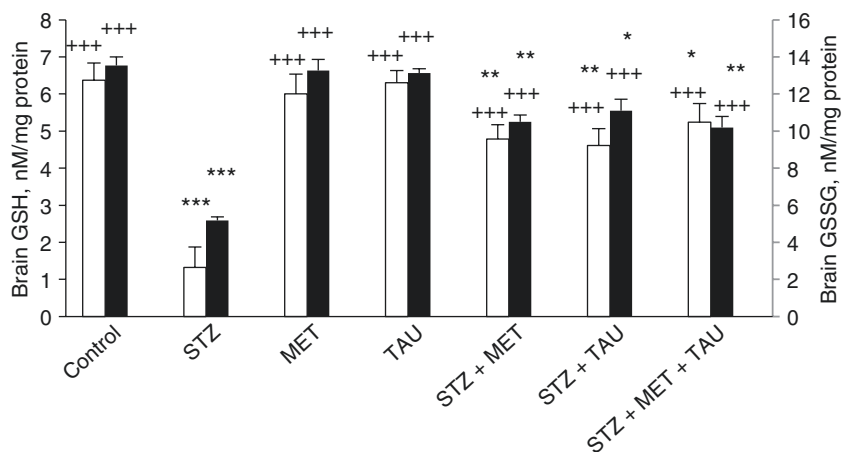
Fig. 2 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain GSH (*open square*) and GSSG (*filled square*) levels of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at *p < 0.05, **p < 0.01 and ***p < 0.001; and from STZ at +++p < 0.001
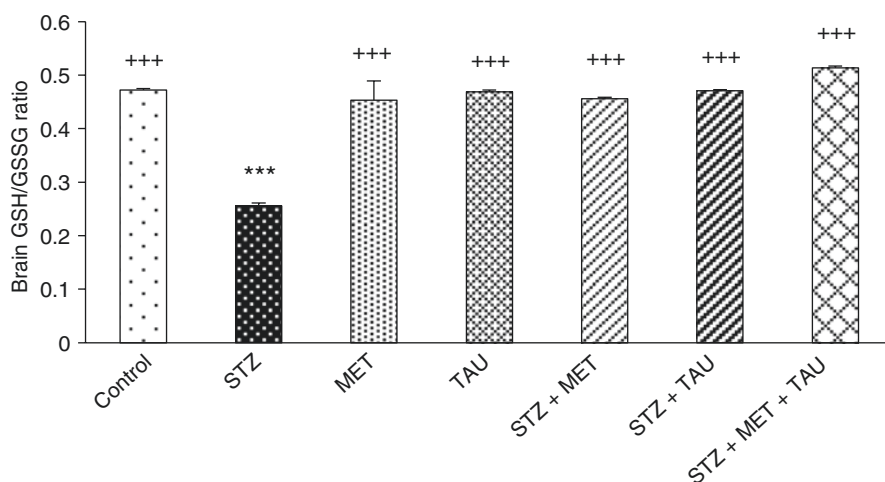

Fig. 3 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain GSH/GSSG ratio of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at ***p < 0.001 and from STZ at +++p < 0.001

combination (-24%, p < 0.01) (Fig. 4). Similarly, diabetes also reduced the brain activity of GST (-51%, p < 0.001), and treatments with MET (-38%, p < 0.001), TAU (-33%, p < 0.001) or MET-TAU (-30%, p < 0.01) were about equipotent in lowering the diabetes-induced decreases (Fig. 4). Moreover, neither MET nor TAU affected the activities of GR and GST in the brain of normal rats.
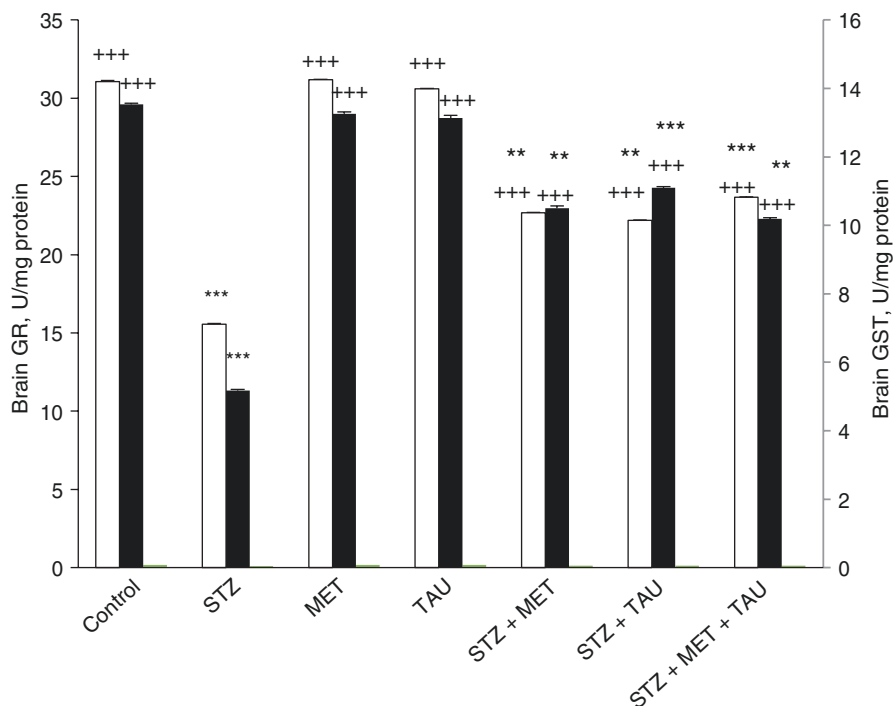
Fig. 4 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain GR (*open square*) and GST (*filled square*) activities of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at $^{**}p < 0.01$ and $^{***}p < 0.001$; and from STZ at $^{+++}p < 0.001$

3.4 Effects on Brain CAT, GPx and SOD Activities

In diabetic rats the activities of CAT (−43%), GPx (−48%) and SOD (−65%) were markedly lower than corresponding activities for normal rats (all at $p < 0.001$) (Figs. 5, 6 and 7, respectively). A daily treatment of the diabetic rats with MET was found to effectively counteract these changes, with the effect being about equal on all three enzymes (−25%, −24% and −24%, respectively, all at $p < 0.01$ vs. control values). An identical treatment with TAU was about equipotent with MET in attenuating the changes of the CAT (−26%, $p < 0.01$, Fig. 5) and GPx (−18%, $p < 0.05$, Fig. 6) activities caused by diabetes but was slightly less potent on the change in SOD activity (−33%, $p < 0.01$, Fig. 7). However, a combined treatment of the diabetic rats with MET plus TAU led to a greater attenuation of the enzymatic changes than either compound alone (−13%, −12% and −18%, respectively, $p < 0.05$, Figs. 5, 6 and 7, respectively). Neither MET nor TAU affected the brain activities of these enzymes in normal rats to a significant extent.
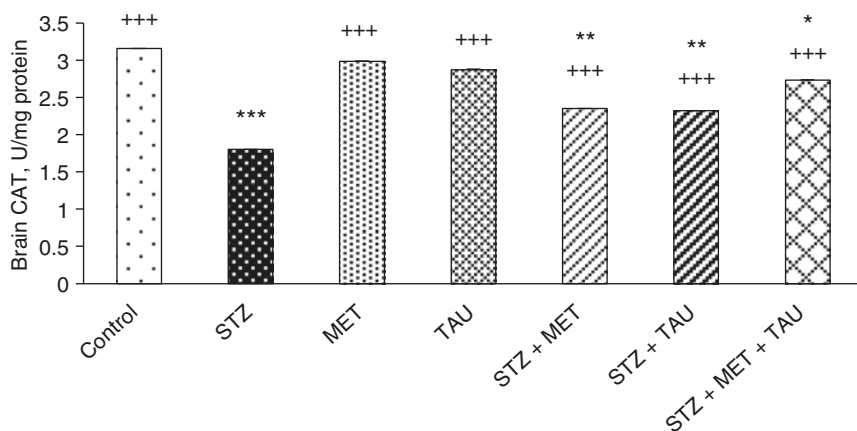
Fig. 5 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain CAT activity of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at $^*p < 0.05$, $^{**}p < 001$ and $^{***}p < 0.001$; and from STZ at $^{+++}p < 0.001$

Fig. 6 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain GPx activity of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and from STZ at $^{+++}p < 0.001$
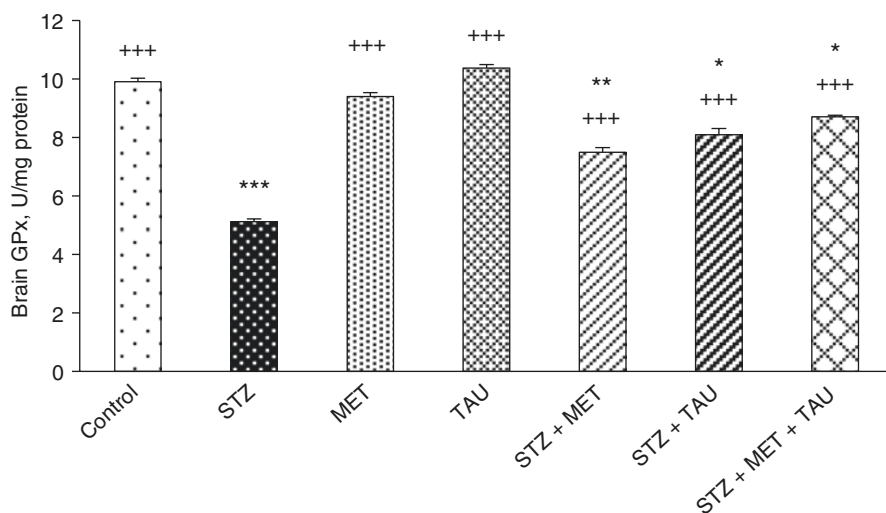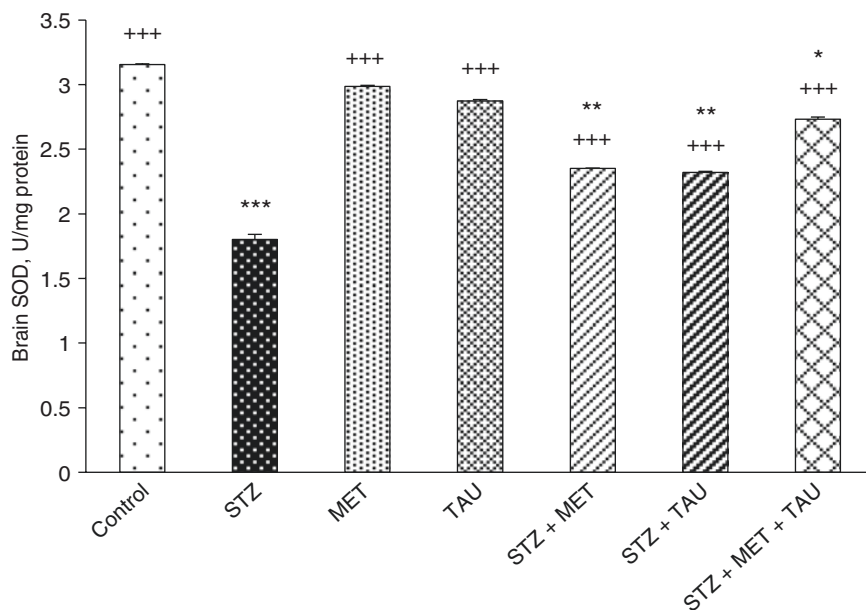
Fig. 7 The effects of a 6 weeks treatment with MET, TAU and MET-TAU on the brain SOD activity of rats made diabetic with STZ. Values are shown as the mean ± SEM for n = 6. Statistical comparisons were significantly different from Control at $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; and from STZ at $^{+++}p < 0.001$

4 Discussion

Based on the results of earlier work on TAU, this conditionally essential β-amino acid has emerged as an attractive candidate for use as a protection against the biochemical and physiological alterations induced by diabetes in mammalian tissues. In addition to a lowering effect on the blood GLC with (Kaplan et al. 2004) or without (Kulakowski and Maturo 1984) an apparent effect on INS secretion, this compound has been reported to increase cell sensitivity to INS and the response of pancreatic β-cells to hyperglycemia (Ribeiro et al. 2009), to attenuate oxidative stress and accompanying INS resistance (Haber et al. 2003), and to inhibit protein glycosylation and formation of advanced glycation end products (Hansen 2001). On the other hand, there is at least one report indicating that TAU does not influence either the blood GLC or the secretion of or sensitivity to INS in humans (Brøns et al. 2004); and another one indicating that the hypoglycemic action of TAU in the rat is only observable in older but not in younger animals (Odetti et al. 2003).

In the particular case of the brain, there is ample evidence to suggest that exogenous TAU can cross the blood brain barrier and enter the brain, where it can protect against oxidative stress in spite of lacking structural features that will allow it to directly interact with H_2O_2, the superoxide radical or the hydroxyl radical (Aruoma et al. 1988). For example, a study by Gürer et al. (2001) found that rats receiving

TAU as part of the drinking water were protected against the decrease of GSH and increase of MDA in the brain of rats fed lead acetate. Furthermore, Mahalakshmi et al. (2003) showed that the consumption of TAU by rats as part of the drinking water was able to counteract the oxidative stress induced by acetonitrile in the brain by reducing LPO, and by enhancing the activities of CAT, GPx, SOD and GST and the concentrations of the antioxidant vitamins C and E. Also, while Yildirim and Kilic (2011) verified that the dosing of young rats with TAU (200 mg/kg) on a daily basis for 7 days protected the cerebellum against the formation of MDA and depletion of GSH brought about by aging, Aydin et al. (2016) reported that the addition of TAU to an animal diet was able to diminish oxidative stress, determined on the basis of MDA, protein carbonyl and GSH levels, and of SOD, GPx, GST activities and apoptosis in the brain of rats subchronically challenged with a daily subcutaneous dose of D-galactose.

This laboratory has verified that in the brain and spinal cord of rats made diabetic with STZ, TAU is able to reverse the increases in MDA and NO concentrations and to effectively protect against the decrease in GSH/GSSG ratio and the losses of CAT, GPx and SOD activities (Patel et al. 2016; Patel and Lau-Cam 2009). On the other hand, Agca et al. (2014) have indicated that TAU was able to partially reduce the serum MDA concentrations and to ameliorate diabetic neuropathy by regulating NF-κB and Nrf2/HO-1 signaling cascades but without altering body weight or blood GLC in diabetic rats, findings that led to the conclusion that in the rat brain TAU reduces oxidative stress through activation of antioxidant defense signaling pathways (Agca et al. 2014).

MET is an established hypoglycemic agent which is not only recommended for intensive glycemic control in type 2 DM but also to achieve improvement in endothelial dysfunction, hemostasis, oxidative stress, INS resistance, lipid profiles, and fat redistribution associated with this disease (Rojas and Gomes 2013). In addition, it can play a role in decreasing the risk of diabetes-related end points in overweight diabetic patients (UK Prospective Diabetes Study Group 1998). Furthermore, a study by Chakraborty et al. (2011), looking at the effects of MET on oxidative stress, nitrosative stress and biomarkers of inflammation in type 2 DM patients, found that this biguanide was able to lower ROS generation, advanced glycation products and pentosidine formation, to enhance total thiol and NO levels, and to restore C reactive protein in the plasma when compared to placebo. The role of MET in modulating oxidative stress has been examined by Esteghamati et al. (2013) in naive human patients with newly diagnosed type 2 diabetes and its effects compared with those of lifestyle modification. After a 3 months treatment, there was a significant reduction in the values of advanced oxidation protein products (AOPP) and advanced glycation end products (AGE), a significant increase in ferritin reducing ability of plasma (FRAP) and paraoxonase activity, and no changes in the value of lecithin-cholesterol acyltransferase, with the AOPP, FRAP and AGE levels changing more significantly with MET than with lifestyle modification alone. In the particular case of the brain, a 4 weeks treatment of Goto-Kakizaki rats, a model of type 2 DM, with TAU was shown to protect against the oxidative imbalance fostered by the diabetic state and manifested by higher levels of MDA, higher activities of

GP and GR but lower of MnSOD relative to corresponding values for nondiabetic Wistar rats (Correia et al. 2008).

Taking into account the reported effects of both TAU and MET on the hyperglycemia, hypoinsulinemia and brain oxidative stress brought about by diabetes, this study was aimed at investigating whether a combined treatment with these two compounds could lead to a greater protection that was possible with MET alone. Thus, diabetic rats on MET showed a greater reduction in circulating GLC levels, an effect that was ~2.5-fold greater than a treatment with TAU. On the other hand, a combined treatment with MET plus TAU enhanced the effect of MET although not to a statistically significant extent. Interestingly, the magnitude of the decrease in circulating GLC by TAU observed in the present study is of the same magnitude as that reported by Winiarska et al. (2009), and has been related, at least in part, to a decreasing effect of this amino acid on gluconeogenesis.

Similarly, both MET and TAU were able to attenuate the decrease of the plasma INS secretion caused by diabetes, with the antagonizing effect of the former being twice as much as that by the latter. In contrast, treating the diabetic rats with MET-TAU led to a smaller decrease in blood INS compared to MET alone. The present results clearly show that while MET shows a strong hypoglycemic effect as a result of positive effect on INS secretion, TAU is a much weaker hypoglycemic and stimulant of INS secretion. Consequently, TAU is found to have enhance the hypoglycemic action of MET to only a limited extent. This effect contrasts markedly with the results of a study by Kaplan et al. (2004) that found TAU to significantly decrease the blood GLC level based on the increase in circulating C-peptide level, an indicator of pancreatic INS secretion. On the other hand, it agrees with the findings of Brøns et al. (2004) in type 2 human diabetics indicating that TAU has no effect on either INS secretion or INS sensitivity. Interestingly, most studies in rodents, including one in which Otsuka Long Evans Tokushima fatty diabetic rats were fed fructose along with a diet supplemented with TAU, have shown both decreased hyperglycemia and INS resistance (Brøns et al. 2004).

MDA is a characteristic product of LPO largely produced *in vivo* through the oxidation of complex lipids, especially long-chain polyunsaturated fatty acids of cellular phospholipids, by oxygen-derived free radicals such as the hydroxyl radical (Slatter et al. 2000). In this case, LPO of cellular structures as a result of free radical activity is thought to play an important role in late complications of diabetes (Kesavulu et al. 2001, 2002). In the present study, diabetic rats showed a very high level of brain MDA at the end of 8 weeks, an effect that was reduced by one-half by both MET and TAU, and to about one-sixth by MET plus TAU.

Furthermore, the marked decrease of the accompanying brain GSH levels (~80%) by diabetes was attenuated to about one-third the control value by either MET or TAU, and to only one-fourth by MET plus TAU. In agreement with the present results, a study by Furfaro et al. (2012) found that supplementation of the drinking water of STZ-treated rats with TAU led after 6 months to attenuation of the loss of hepatic GSH and of the increase in GSSG/GSH ratio, possibly as a result of a more efficient GSH synthesis and decreased GSH loss and degradation. A further consequence of the increase in brain GSH by MET, TAU or their combination

may be responsible for the significant increase in the activity of GR, the enzyme that mediates the regeneration of GSH from its disulfide form GSSG. While these compounds almost double the brain GR activity of diabetic rats, a combined treatment did not increase the activity further over that seen with the individual treatments. However, as expected, the increase of the GSH levels by either MET or TAU was accompanied by an increase of the activity of the selenium-dependent GPx, viewed as a protection against lipid peroxide and H_2O_2 formation (Arthur 2000) as well as from hydroxyl radicals formed via iron-catalyzed Fenton-type reactions from H_2O_2 by reducing this oxidant to water (Barlow-Walden et al. 1995). In addition, while diabetes lowered the activity of GST, an enzyme conjugating GSH with a variety of electrophile and demonstrating GSH peroxidase activity (Gronwald and Plaisance 1998), by one-half of the control activity, MET, and to a slightly greater extent, TAU, were able to reduce the loss by at least 25%, an effect that was further increased when MET and TAU were given together. A previous study by Lim et al. (1998) using KK mice, a spontaneous hyperglycemic animal mode of type 2 diabetes, found that TAU was able to increase the hepatic activity of GPx but without effecting that of GST.

Furthermore, the present study found that both MET and TAU were about equipotent in almost doubling the activity of the brain GR, the enzyme that mediates the regeneration of GSH from its disulfide form, in diabetic rats, with a combined treatment providing an insignificantly better effect than either compound alone. A similar result for TAU was obtained by Winiarska et al. (2009) in the kidney of alloxan-treated rabbits consuming TAU as part of the drinking water. In general, it would appear that the effects of TAU on the activities of GR and GPx are dependent on the site examined, with the activity of the former showing a greater variability than that of the latter (Anand et al. 2011).

The activities of both CAT and SOD were markedly decreased in the brain of diabetic rats, with the decrease being greater for CAT than for SOD. A treatment with either MET or TAU led to about a one-half reduction of the decrease, an effect that was further enhanced when the two compounds were given together. A similar trend was noted in this laboratory in the brain of STZ-treated rats at 24 h after they had received an i.p. dose of TAU (Patel and Lau-Cam 2009; Patel et al. 2016). On the other hand, a reduction in these two enzyme activities in type 2 DM has been previously reported for the serum (Obi et al. 2016) and, to a lesser extent, brain (Aluwong et al. 2016) of alloxan-treated rats.

5 Conclusion

The present results indicate that although MET and TAU may differ in their intrinsic effects on the circulating GLC and INS levels of rats made diabetic with STZ, they, however, share similar effects on the accompanying oxidative stress that develops in the brain. More importantly, adding TAU to a treatment with MET will enhance the attenuating actions of MET on diabetes-induced oxidative stress in the brain.

References

Acar A, Akil E, Alp H, Evliyaoglu O et al (2012) Oxidative damage is ameliorated by curcumin treatment in brain and sciatic nerve of diabetic rats. Int J Neurosci 122:367–372

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Agca CA, Tuzcu M, Hayirli A, Sahin K (2014) Taurine ameliorates neuropathy via regulating NF-κB and Nrf2/HO-1 signaling cascades in diabetic rats. Food Chem Toxicol 71:116–121

Aluwong T, Ayo JO, Kpukple A, Oladipo OO (2016) Amelioration of hyperglycaemia, oxidative stress and dyslipidaemia in alloxan-induced diabetic Wistar rats treated with probiotic and vitamin C. Nutrients 8:151

Anand P, Rajakumar D, Jeraud M, Felix AJ, Balasubramanian T (2011) Effects of taurine on glutathione peroxidase, glutathione reductase and reduced glutathione levels in rats. Pak J Biol Sci 14:219–225

Arthur JR (2000) The glutathione peroxidases. Cell Mol Life Sci 57:1825–1835

Aruoma OI, Halliwell B, Hoey BM, Butler J (1988) The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J 256:251–255

Aydin AF, Çoban J, Doğan-Ekici I et al (2016) Carnosine and taurine treatments diminished brain oxidative stress and apoptosis in D-galactose aging model. Metab Brain Dis 31:337–345

Baquer NZ, Hothersall JS, McLean P, Greenbaum AL (1990) Effect of aging on soluble and membrane bound enzymes in rat brain. Neurochem Int 16:369–375

Barlow-Walden LR, Reiter RJ, Abe M et al (1995) Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int 26:49–78

Bonnefont-Rousselot D (2002) Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care 5:561–568

Brøns C, Spohr C, Storgaard H, Dyerberg J, Vaag A (2004) Effect of taurine treatment on insulin secretion and action, and on serum lipid levels in overweight men with a genetic predisposition for type II diabetes mellitus. Eur J Clin Nutr 58:1239–1247

Budhram R, Pandya KG, Lau-Cam CA (2013) Protection by taurine and thiotaurine against biochemical and cellular alterations induced by diabetes in a rat model. Adv Exp Med Biol 775:321–343

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Cardoso S, Santos RX, Correia SC et al (2013) Insulin-induced recurrent hypoglycemia exacerbates diabetic brain mitochondrial dysfunction and oxidative imbalance. Neurobiol Dis 49:1–12

Chakraborty A, Chowdhury S, Bhattacharyya M (2011) Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. Diabetes Res Clin Pract 93:56–62

Correia S, Carvalho C, Santos M et al (2008) Metformin protects the brain against the oxidative imbalance promoted by type 2 diabetes. Med Chem 4:358–364

Delides A, Spooner RJ, Goldberg DM, Neal FE (1976) An optimized semiauto-matic rate method for serum glutathione reductase activity and its application to patients with malignant disease. J Clin Pathol 29:73–77

Esteghamati A, Eskandari D, Mirmiranpour H et al (2013) Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. Clin Nutr 32:179–185

Furfaro AL, Nitti M, Marengo B et al (2012) Impaired synthesis contributes to diabetes-induced decrease in liver glutathione. Int J Mol Med 29:899–905

Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. Circ Res 107:1058–1070

Gronwald JW, Plaisance KL (1998) Isolation and characterization of glutathione S-transferase isozymes from sorghum. Plant Physiol 117:877–892

Güntherberg H, Rost J (1966) The true oxidized glutathione content of red blood cells obtained by new enzymic and paper chromatographic methods. Anal Biochem 15:205–210

Günzler WA, Flohé L (1985) Glutathione peroxidase. In: Greenwald RA (ed) CRC handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL, pp 285–290

Gürer H, Özgünes H, Saygin E, Ercal N (2001) Antioxidant effect of taurine against lead-induced oxidative stress. Arch Environ Contam Toxicol 41:397–402

Haber CA, Lam TK, Yu Z et al (2003) N-Acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance in vivo: possible role of oxidative stress. Am J Physiol Endocrinol Metab 285:E744–E753

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Hansen SH (2001) The role of taurine in diabetes and the development of diabetic complications. Diabetes Metab Res Rev 17:330–346

Hissin PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74:214–226

Kaplan B, Karabay G, Zağyapan RD et al (2004) Effects of taurine in glucose and taurine administration. Amino Acids 27:327–333

Kesavulu MM, Rao BK, Giri R et al (2001) ACH. Lipid peroxidation and antioxidant enzyme status in type 2 diabetics with coronary heart disease. Diabetes Res Clin Pract 53:33–39

Kesavulu MM, Kameswararao B, Apparao C, Kumar EG (2002) Effect of omega-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. Diabetes Metab 28:20–26

Kulakowski EC, Maturo J (1984) Hypoglycemic properties of taurine: not mediated by enhanced insulin release. Biochem Pharmacol 33:2835–2838

Kumar JS, Menon VP (1993) Effect of diabetes on levels of lipid peroxides and glycolipids in rat brain. Metabolism 42:1435–1439

Lim E, Park S, Kim H (1998) Effect of taurine supplementation on the lipid peroxide formation and the activities of glutathione-related enzymes in the liver and islet of type I and II diabetic model mice. Adv Exp Med Biol 442:99–103

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Mahalakshmi K, Pushpakiran G, Anuradha CV (2003) Taurine prevents acrylonitrile-induced oxidative stress in rat brain. Pol J Pharmacol 55:1037–1043

Maritim AC, Sanders RA, Watkins JB 3rd (2003) Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 17:24–38

Mastrocola R, Restivo F, Vercellinatto I et al (2005) Oxidative and nitrosative stress in brain mitochondria of diabetic rats. J Endocrinol 187:37–44

Misra HP (1985) Adrenochrome assay. In: Greenwald RA (ed) CRC handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL, pp 237–241

Muriach M, Flores-Bellver M, Romero FJ, Barcia JM (2014) Diabetes and the brain: oxidative stress, inflammation, and autophagy. Oxidative Med Cell Longev 2014:102158

Obi BC, Okoye TC, Okpashi VE, Igwe CN, Alumanah EO (2016) Comparative study of the antioxidant effects of metformin, glibenclamide, and repaglinide in alloxan-induced diabetic rats. J Diabetes Res 2016:1635361

Odetti P, Pesce C, Traverso N et al (2003) A comparative trial of N-acetylcysteine, taurine, and oxerutin on skin and kidney damage in long-term experimental diabetes. Diabetes 52: 499–505

Ortiz-Avila O, Esquivel-Martínez M, Olmos-Orizaba BE et al (2015) Avocado oil improves mitochondrial function and decreases oxidative stress in brain of diabetic rats. J Diabetes Res 2015:485759

Pandya KG, Budhram R, Clark G, Lau-Cam CA (2013) Comparative evaluation of taurine and thiotaurine as protectants against diabetes-induced nephropathy in a rat model. Adv Exp Med Biol 775:371–394

Patel S, Lau-Cam CA (2009) Antioxidant actions of taurine and structurally related compounds in the brain of diabetic rats. Abstract 1555. Society of Toxicology Annual Meeting, Baltimore

Patel SN, Pandya K, Clark GJ, Parikh MC, Lau-Cam CA (2016) Comparison of taurine and pantoyltaurine as antioxidants *in vitro* and in the central nervous system of diabetic rats. Exp Toxicol Pathol 68:103–112

Ribeiro RA, Bonfleur ML, Amaral AG et al (2009) Taurine supplementation enhances nutrient-induced insulin secretion in pancreatic mice islets. Diabetes Metab Res Rev 25:370–379

Rojas LBA, Gomes MB (2013) Metformin: an old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr 5:6

Slatter DA, Bolton CH, Bailey AJ (2000) The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia 43:550–557

UK Prospective Diabetes Study (UKPDS) Group (1998) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 352:854–865

Vincent AM, Russell JW, Low P, Feldman EL (2004) Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev 25:612–628

Wang X, Michaelis EK (2010) Selective neuronal vulnerability to oxidative stress in the brain. Front Aging Neurosci 2:12

Wang X, Wang W, Li L et al (2014) Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim Biophys Acta 1842:1240–1247

Winiarska K, Szymanski K, Gorniak P et al (2009) Hypoglycaemic, antioxidative and nephroprotective effects of taurine in alloxan diabetic rabbits. Biochimie 91:261–270

Wollenberger A, Ristau O, Schoffa G (1960) Ein einfache technik die extrem schnellen Abkühlung grüsserer Gewebestücke. Pfluegers Arch 270:399–412

Yildirim Z, Kilic N (2011) Effects of taurine and age on cerebellum antioxidant status and oxidative stress. Int J Gerontol 5:166–170