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ORIGINAL PAPER

Reduced Brain Antioxidant Capacity in Rat Models of Betacytotoxic-Induced Experimental Sporadic Alzheimer's Disease and Diabetes Mellitus

Ismet Tahirovic · Emin Sofic · Aida Sapcanin · Ismet Gavrankapetanovic · Lidija Bach-Rojecky · Melita Salkovic-Petrisic · Zdravko Lackovic · Siegfried Hoyer · Peter Riederer

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Abstract It is believed that oxidative stress (OS) plays a central role in the pathogenesis of metabolic diseases like diabetes mellitus (DM) and its complications (like peripheral neuropathy) as well as in neurodegenerative disorders like sporadic Alzheimer's disease (sAD). Representative experimental models of these diseases are streptozotocin (STZ)-induced diabetic rats and STZ-intracerebroventricularly (STZ-icv) treated rats, in which antioxidant capacity (AC) against peroxyl ($ORAC_{-ROO^{\bullet}}$) and hydroxyl ($ORAC_{-OH^{\bullet}}$) free radicals (FR) was measured in three different brain regions: the hippocampus (HPC), the cerebellum (CB), and the brain stem (BS) by means of oxygen radical absorbance capacity (ORAC) assay. In the brain of both STZ-induced diabetic and STZ-icv treated rats decreased AC has been found demonstrating regionally specific distribution. In the diabetic rats these abnormalities were not associated with the development of peripheral diabetic neuropathy (PDN). Also, these abnormalities were not prevented by the intracerebroventricularly (icv) pretreatment of glucose transport inhibitor 5–thio-D-glucose (TG) in the STZ-icv treated rats, suggesting different mechanism of STZ-induced central effects from those at the periphery. Similarities of the OS alterations in the brain of STZ-icv rats and humans with sAD could be useful in the search for the new drugs in the treatment of sAD that have antioxidant activity. In the STZ-induced diabetic animals the existence of PDN was tested by the paw pressure test, 3 weeks following the diabetes induction. Mechanical nociceptive thresholds were measured three times at 10–min intervals by applying increased pressure to the hind paw until the paw-withdrawal or overt struggling was elicited. Only those diabetic animals which demonstrated decreased withdrawal threshold values in comparison with the control non-diabetic animals (C) were considered to have developed the PDN.

Keywords Rat · Streptozotocin · Diabetes mellitus · Antioxidant capacity · Oxidative stress

Abbreviations

AAPH	2,2′-Azobis (2–amidino-propane) dihydrochloride
AC	Antioxidant capacity
AD	Alzheimer's disease
AL	Alloxan
AUC	Area under curve
BCT	Betacytotoxic

Special issue dedicated to Dr. Moussa Youdim.

I. Tahirovic · E. Sofic (✉) · A. Sapcanin
Department of Chemistry, Faculty of Science, University of Sarajevo, Zmaja od Bosne 35, 71 000 Sarajevo, Bosnia and Herzegovina
e-mail: esofic@pmf.unsa.ba

I. Gavrankapetanovic
School of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

L. Bach-Rojecky
Department of Pharmacology, School of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

M. Salkovic-Petrisic · Z. Lackovic
Department of Pharmacology and Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia

S. Hoyer
Department of Pathology, University Clinic, University of Heidelberg, Heidelberg, Germany

P. Riederer
Clinical Neurochemistry, Clinic and Policlinic for Psychiatry, University of Wuerzburg, Wuerzburg, Germany

β-PE	Beta-phycoerythrin
BS	Brain stem
BSA	Albumin
C	Control non-diabetic animals
CB	Cerebellum
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
f.c.	Final concentration
FL	Fluorescein
FR	Free radicals
GLUT2	Glucose transporter two
HPC	Hippocampus
icv	Intracerebroventricularly
ip	Intraperitoneal
LC/MS	Liquid chromatography/mass spectrometry
ORAC	Oxygen radical absorbance capacity
$ORAC_{-ROO^{\bullet}}$	Antioxidant capacity against peroxyl free radicals
$ORAC_{-OH^{\bullet}}$	Antioxidant capacity against hydroxyl free radicals
OS	Oxidative stress
PDN	Peripheral diabetic neuropathy
ROS	Reactive oxygen species
sAD	Sporadic Alzheimer's disease
sc	Subcutaneous
STZ	Streptozotocin
Trolox	6–Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid
TE	Trolox equivalents
TG	5-Thio-D-glucose

Introduction

The free radicals (FR) hypothesis of metabolic diseases and neurodegeneration suggests that pathological disturbances occur as a result of an increasing inability to cope with oxidative stress (OS). Oxidative stressors induce an imbalance between oxidants and antioxidants in favor of the former leading to the oxidative damage of the molecules like deoxyribonucleic acid (DNA), lipids and proteins [1, 2]. OS plays an important role in the pathogenesis of diabetes mellitus (DM) and its complications, microangiopathy and associated neuropathy in particular [1]. Hyperglycemia induces both the disruption of mitochondrial membrane potential, and an increase in reactive oxygen species (ROS) which are capable of decreasing the antioxidant status in man [3, 4]. In line with that, decreased antioxidant capacity (AC) has been found in the serum of patients with diabetic polyneuropathy in comparison to the control (non-diabetic) patients, as measured by oxygen radical absorbance capacity (ORAC) assay [1, 5]. Additionally, it has been demonstrated that both in diabetic humans and experimentally diabetic rats, OS seems to play an important role in the brain damage [6]. Increased hydroxyl radical ($OH^{\bullet}$) formation as well as increased levels of free fatty acids and malondialdehyde and decreased activities of antioxidative enzymes catalase and superoxide dismutase have been found in the brain of experimentally diabetic rats [7, 8]. Investigation of the brain AC in relation to the peripheral diabetic neuropathy (PDN) has not been reported.

Experimental DM in animals is usually produced by betacytotoxic (BCT) drugs streptozotocin (STZ) and alloxan (AL) which given parentherally in high doses damage pancreatic beta cells and decrease insulin production/secretion [9]. Central, intracerebroventricular (icv) administration of low STZ and AL doses, respectively, does not produce DM in rats, but produces regionally specific brain neurochemical changes that are, in general, similar to those found in BCT-induced diabetes [10–13]. Because of induction of the long-term and progressive cognitive deficits and decreased brain glucose and energy metabolism [14, 15], STZ-intracerebroventricularly (STZ-icv) treated rats have been proposed as an experimental model of sporadic Alzheimer's disease (sAD) in which the existence of the brain type of non-insulin dependent diabetes («cerebral diabetes») has been suggested [16]. In addition to that, Alzheimer's like alterations in the insulin receptor signaling cascade in the brain have been reported recently in the STZ-icv treated rats [17]. This gives a further support to the hypothesis that this model is the representative experimental model of sAD which, contrary to the transgenic Tg 2756 mice model of Alzheimer's disease (AD) [18], is not based on the gene manipulations. STZ treatment has been reported to generate ROS in addition to release of nitric oxide [9]. In line with that, significant elevation of malondialdehyde and decrement of glutathione levels have been found in the brain of STZ-icv treated rats, supporting the hypothesis of OS development [19, 20]. This is in agreement with the report of FR and OS involvement in the pathophysiology of AD [21].

The present study, was aimed to investigate the AC in the different rat brain regions following the central (icv) non-diabetogenic and peripheral diabetogenic treatment of BCT drugs in the conditions of present or absent PDN, as well as following the icv-treatment of glucose transport inhibitor alone or combined with STZ-icv treatment, by means of ORAC method involving two different FR generators [2, 5, 22–26], modified by Sofic et al. [27]. ORAC method has an advantage over other assays, because this method utilizes an area-under-curve technique and thus combines both inhibition time and inhibition degree of FR action by an antioxidant into a single quantity. Fluorescein (FL) was used as a target of FR attack, with 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) as a peroxyl

radical ($ROO^{\bullet}$) generator, and hydrogen peroxide with cupric sulfate penta hydrate as a $OH^{\bullet}$ generator. $ROO^{\bullet}$ is a common FR found in the body and used in the antioxidant activity assays [22, 23, 28–30]. It is slightly less reactive than $OH^{\bullet}$ and thus possesses an ''extended'' half-life of seconds instead of nanoseconds [31]. In original developed automated method beta-phycoerythrin (β-PE), a protein from *Porphyridium cruentum*, was used as a target of FR attack [2, 5, 22–26]. Using the ORAC assay with β-PE, Cao et al. demonstrated that the same flavonoids could behave as both antioxidants and prooxidants, depending on concentration and FR source [25]. They reported that flavonoids acted as antioxidants against FR, i.e., $ROO^{\bullet}$ and $OH^{\bullet}$ but demonstrated prooxidant activity when a transition metal (Cu^{2+}) was available. An improved ORAC method has been developed and validated using FL as the photo sensor. Ou et al. demonstrated that FL is superior to β-PE [32]. The oxidized FL products with $ROO^{\bullet}$ were identified by liquid chromatography/mass spectrometry (LC/MS) system, and the reaction mechanism was determined.

Materials and methods

Chemicals

Albumin (BSA), lyophilized powder was purchased from Sigma GmbH, Deisenhofen, Germany; FL, Standard Fluka for fluorescence–free acid was obtained from Fluka Chemie GmbH, Steinheim, Germany; AAPH, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), STZ and alloxan monohydrate (AL) were purchased from Sigma Aldrich Chemie GmbH; 5-thio-D-glucose (TG) was purchased from BioChemika. Cupric sulfate penta hydrate and hydrogen peroxide were obtained from Kemika, Zagreb, Croatia.

Animals

Adult male, 2–4 month-old-Wistar rats (Department of Pharmacology, School of Medicine, University of Zagreb, Croatia) were used throughout the study. In all experiments including those with diabetes induction all animals were kept on standardized food pellets and water ad libitum.

Treatments

Intracerebroventricular drug administration

Rats were randomly divided in four groups (5–6 per group) and given general anaesthesia (chloralhydrate 300 mg/kg, ip), followed by injection of different drugs icv bilaterally into the lateral ventricle (2 μL/ventricle), according to the procedure described by Noble et al. [33]. The following drug treatments were applied in a single dose: (1) STZ (1 mg/kg, dissolved in 0.05 M citrate buffer pH 4.5); (2) TG (375 μg/kg, dissolved in the same vehicle as STZ); (3) TG (375 μg/kg) + STZ (1 mg/kg); (4) an equal volume of vehicle (controls). Animals were sacrificed 3 months after the drug icv treatment. The brains were quickly removed, the hippocampus (HPC), the cerebellum (CB) and the brain stem (BS) cut out, immediately frozen and stored at -80°C. STZ-icv treated animals had no symptoms of diabetes and steady-state blood glucose level did not differ in comparison with control animals Control non-diabetic animals (C).

Diabetes induction

Experimental diabetes was induced by a single intraperitoneal (ip) injection of STZ (70 mg/kg, dissolved in 0.05 M citrate buffer pH 4.5) or a single subcutaneous (sc) injection of AL monohydrate (150 mg/kg, dissolved in saline) [9]. The corresponding C were treated with an equal volume of vehicle ip or sc, respectively. Animals which developed polydipsia, polyuria, and polyphagia, and had blood glucose levels >20 mmol/l after 1 week were used for the experiments. STZ- and AL-induced diabetic animals and corresponding C were sacrificed 10 and 21 weeks following the diabetes induction, respectively.

Measurement of pain reactivity, i.e., PDN

In the STZ-induced diabetic animals the existence of PDN was tested by the paw pressure test by [34, 35] 3[t1] weeks following the diabetes induction. Mechanical nociceptive thresholds were measured three times at 10-min intervals by applying increased pressure to the hind paw until the paw-withdrawal or overt struggling was elicited. Only those diabetic animals which demonstrated decreased withdrawal threshold values in comparison with the C were considered to have developed the PDN (Fig. 2).

Sample preparation

The crude tissue extracts from the rat brain HPC, BS, and CB were prepared by homogenizing the tissues in a 75 mM phosphate buffer pH 7.3 (10 ml buffer per gram of tissue). The homogenates were centrifugated and separation of the soluble fractions was performed by two-step centrifugation (15,000 rpm 30 min, and 15,000 rpm 10 min at 4°C). The supernatant was ready for analysis after appropriate dilution with the buffer solution.

Oxygen radical absorbance capacity (ORAC) assay

The assay is based on the propensity of the fluorescence emitted by the protein β-PE from porphyridium cruentum or FL to be quenched when exposed to FR action. This assay utilizes an area-under curve technique thus combining both inhibition time and inhibition degree of FR action by an antioxidant into a single quantity.

All ORAC analysis were performed on a Perkin Elmer spectrometer LS 55 with a fluorescent filters (Ex: 485 nm; Em: 520 nm) [2]. Diluted supernatant (1:50) was used for the analysis. The final assay mixture (2.00 ml total volume) was prepared by adding of 50 µl 0.42 µM FL [10.50 nM final concentration (f.c.)], 100 µl biological sample, and 1,800 µl phosphate buffer pH 7.30. This mixture was thermostated at 37°C for 15 min, after which the following was added: 50 µl 640 mM AAPH (16 mM f.c.) as a peroxyl radical generator ($ORAC_{-ROO^{\bullet}}$ assay) or 25 µl 7.20 mM Cu^{2+} (90 µM f.c.) and then 25 µl 9.06 M H_2O_2 (113.30 mM f.c.) as mainly a hydroxyl radical generator ($ORAC_{-OH^{\bullet}}$ assay). The intensity of relative fluorescence was measured every 10 min up to 180 min. Standard solution was Trolox, a water-soluble vitamin E analog (1 µM). Blank solution was only FL buffer solution with FR generator. The spectrofluorometer was programmed to record the fluorescence of FL every 10 min after AAPH, and Cu^{2+} then H_2O_2 were added for as long as 180 min and the samples were thermostated at 37°C (KP 20-Lauda). All fluorescence measurements were expressed relative to the initial reading. The calculation of ORAC values and the decay curve of FL are presented in Fig. 1a and b. The ORAC value is obtained by measuring the net protection area (S) under the quenching curve of FL or β-PE in the presence of the antioxidant. The area under curve (AUC) for the blank is subtracted from the AUC for the samples containing antioxidant.

In this work, final results were calculated using the differences of areas under the FL decay curves between the blank and a sample and expressed as a µmol standard equivalents per g of fresh tissue or µmol per mg of protein:

$$\mathrm{ORAC}_{(\mathrm{TE},\,\mu\mathrm{mol/g})} = k \cdot \frac{(S_S - S_B)}{(S_{St} - S_B)}. \quad (1)$$

ORAC—oxygen radical absorbance capacity expressed as trolox equivalents (TE, µmol/g), k—dilution factor, S_S—integrated area under decay fluorescent curve for sample solution, S_B—for blank, S_{st}—for standard.

Integrated area under decay fluorescent curve for sample, standard or blank is calculated by formula of trapeze area [36] as follows:

$$S = \frac{1}{2}\left[\left(t_1 - t_0\right)\cdot\left(f_1 + f_0\right) + \left(t_2 - t_1\right)\cdot\left(f_2 + f_1\right) + \cdots + \left(t_{i+1} - t_i\right)\cdot\left(f_{i+1} + f_i\right)\right]. \quad (2)$$

S—integrated area under decay fluorescent curve for sample, blank or standard, t—incubation time, f—relative fluorescence intensity.

Protein assay

Total protein content was determined using modified Biuret reaction. This method is based on measurements of the absorbance of Cu(II)-protein complex at 545 nm [37].

Statistical analysis

Data was sent electronically from the Perkin-Elmer spectrometer LS 55 to a PC system running FL WinLab software (Perkin-Elmer, Boston, MA, USA). Differences between the C and treated groups were calculated by the Student's t-test.

Ethics

The animal treatments were carried out in Croatia, and were under the guidance of the Principles of Laboratory Animal care (NIH Publication No. 86–23, revised in 1985),

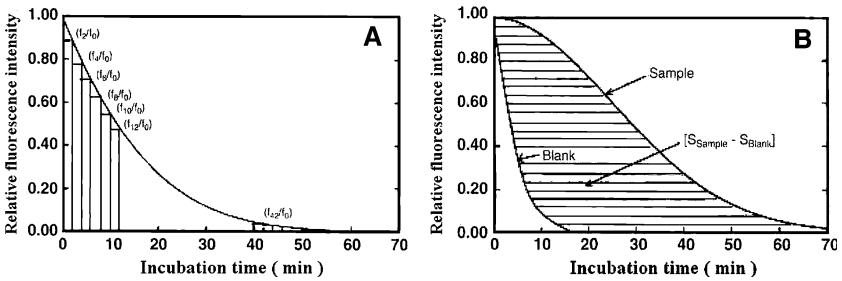

Fig. 1 The calculation of ORAC values: (**A**) relative fluorescence of β-PE or FL at different incubation time points, where f_0 initial fluorescence, f_2 fluorescence at 2 min, etc.; (**B**) ORAC value, calculated as $\left(S_{\mathrm{sample}} - S_{\mathrm{blank}}\right)/\left(S_{\mathrm{trolox}} - S_{\mathrm{blank}}\right)$

according to the Croatian Act on Animal Welfare (NN19; 1999) and were approved by The Ethics Committee of the Zagreb University School of Medicine (No. 04–1343–2006).

Results

Measurements of the $ORAC_{-ROO}^{\bullet}$ and the $ORAC_{-OH}^{\bullet}$ in three different brain regions of the control rats treated with a vehicle only, demonstrated that both the $ORAC_{-ROO}^{\bullet}$ and the $ORAC_{-OH}^{\bullet}$ values were the highest in the CB in comparison to the significantly lower levels found in the BS and the HPC (Table 1).

Decreased $ORAC_{-OH}^{\bullet}$ and $ORAC_{-ROO}^{\bullet}$ has been found in the brain of experimentally diabetic rats. Significantly decreased $ORAC_{-ROO}^{\bullet}$ values were found in the CB of the STZ-induced diabetic rats in comparison with C, regardless whether the PDN had been manifested or not (Table 2).

Table 1 Antioxidant capacity against hydroxyl ($ORAC_{-OH}^{\bullet}$) and peroxyl ($ORAC_{-ROO}^{\bullet}$) FR in different brain regions of the control rats

Region	$ORAC_{-OH}^{\bullet}$ (μmol/g fresh tissue)	$ORAC_{-ROO}^{\bullet}$ (μmol/g fresh tissue)
CB (9)	1,664.0 ± 343.5	4,555.3 ± 249.2
BS (10)	455.6 ± 250.2*	3,213.6 ± 293.9**
HPC (4)	660.5 ± 60.4*	1,906.2 ± 212.3***

Data are expressed as mean ± SD; number of animals is given in parenthesis

ORAC oxygen radical absorbance capacity assay, *CB* cerebellum, *BS* brain stem, *HPC* hippocampus

*$p < 0.05$

**$p < 0.03$

***$p = 0.04$ versus cerebellum by Student's *t*-test

Interestingly, decreased $ORAC_{-ROO}^{\bullet}$ values in the CB were found also in animals which were treated with the diabetogenic dose of STZ but did not develop hyperglycemia and diabetes (Table 2). A tendency of decreased $ORAC_{-OH}^{\bullet}$ in the CB was also observed in the STZ-induced diabetic rats with, or without manifested neuropathy but the difference was not statistically significant and no change in the AC against both FR has been found in the BS as well (Table 2). In the AL-induced diabetic rats decrement in the brain AC was more pronounced against the $OH^{\bullet}$ than the $ROO^{\bullet}$ FR, the difference being statistically significant in the CB, the BS, and the HPC, respectively, while significantly decreased $ORAC_{-ROO}^{\bullet}$ were found in the BS of the AL-induced diabetic rats only (Table 3).

Paw-pressure test for detection of neuropathy in STZ-induced diabetic rats illustrated in Fig. 2.

In comparison to the C, significantly decreased $ORAC_{-OH}^{\bullet}$ was found in the CB and the BS of the STZ-icv treated rats while $ORAC_{-ROO}^{\bullet}$ remained unchanged (Table 4). In the TG-icv treated rats decrement of the AC was even more pronounced since significantly decreased $ORAC_{-ROO}^{\bullet}$ levels were found in the HPC and significantly decreased $ORAC_{-OH}^{\bullet}$ levels were found in all three investigated brain regions in comparison to the C (Table 4). Interestingly, similar decrement of the brain AC was found following the combined TG + STZ-icv treatment, i.e., the values of $ORAC_{-OH}^{\bullet}$ were also significantly decreased in all three investigated brain regions in comparison to the C ones, while those of $ORAC_{-ROO}^{\bullet}$ remained unchanged (Table 4).

Discussion

Diabetics and experimental animal models exhibit high OS due to persistent and chronic hyperglycemia [6]. A close

Table 2 Antioxidant capacity against hydroxyl ($ORAC_{-OH}^{\bullet}$) and peroxyl ($ORAC_{-ROO}^{\bullet}$) FR in different brain regions of the STZ-induced diabetic (10 weeks) rats with or without manifested PDN

Region	Treatment	$ORAC_{-OH}^{\bullet}$ (μmol/g fresh tissue)	$ORAC_{-ROO}^{\bullet}$ (μmol/g fresh tissue)
CB	C (4)	2,474.6 ± 1,747.6	6,261.7 ± 2,360.5
	STZ - D (4)	2,740.3 ± 1,705.0	3,230.1 ± 1,595.0*
	STZ + D (5)	1,932.2 ± 592.9	3,156.8 ± 383.7*
	STZ + Dn (5)	1,675.0 ± 721.3	5,605.9 ± 1,840.4*
BS	C (4)	216.9 ± 162.7	3,637.0 ± 826.6
	STZ - D (4)	494.4 ± 434.1	2,866.0 ± 1,156.8
	STZ + D (5)	360.4 ± 201.7	3,402.3 ± 1,136.8
	STZ + Dn (5)	341.6 ± 136.1	3,376.6 ± 443.5

Data are expressed as mean ± SD; number of animals is given in parenthesis

ORAC oxygen radical absorbance capacity assay, *CB* cerebellum, *BS* brain stem, *HPC* hippocampus, *ND* no data, *C* control group, *STZ - D* group treated parentherally with a diabetogenic streptozotocin dose which did not become diabetic, *STZ + D* streptozotocin-induced diabetic group which did not develop peripheral neuropathy, *STZ + Dn* streptozotocin-induced diabetic group which developed peripheral neuropathy

*$p < 0.05$ versus control group by Student's *t*-test

Table 3 Antioxidant capacity against hydroxyl ($ORAC_{-OH}^{\bullet}$) and peroxyl ($ORAC_{-ROO}^{\bullet}$) FR in different brain regions of the AL-induced diabetic (21 weeks) rats

Region	Treatment	$ORAC_{-OH}^{\bullet}$ (μmol/g fresh tissue)	$ORAC_{-ROO}^{\bullet}$ (μmol/g fresh tissue)
CB	C (4)	510.3 ± 219.2	2,061.3 ± 182.0
	AL + D (4)	432.4 ± 206.3***	2,117.7 ± 265.3
BS	C (3)	584.4 ± 163.5	1,737.4 ± 268.4
	AL + D (5)	435.3 ± 164.2**	1,479.9 ± 129.9*
HPC	C (4)	660.5 ± 60.4	1,906.2 ± 212.3
	AL + D (4)	595.3 ± 61.8****	2,066.7 ± 347.0

Data are expressed as mean ± SD; number of animals is given in parenthesis

ORAC oxygen radical absorbance capacity assay, *CB* cerebellum, *BS* brain stem, *HPC* hippocampus, *C* control group, *AL + D* alloxan-induced diabetic group

*$p < 0.05$

**$p < 0.04$

***$p < 0.03$

****$p = 0.02$versus control group by Student's *t*-test

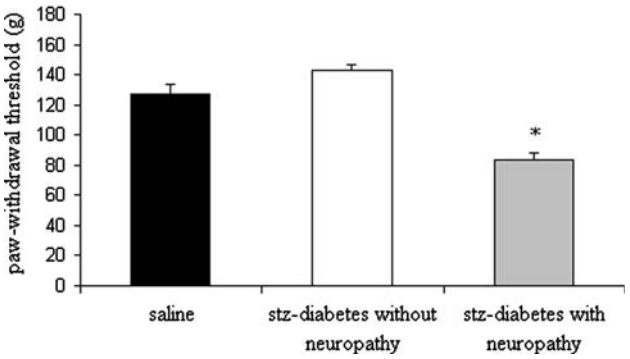

Fig. 2 Sensitivity to mechanical stimuli measured by the paw-pressure test. Measurements were done 3 weeks following the streptozotocin (70 mg/kg, i.p.) injection. Results are presented as mean ± SE, $N = 6$. *$p < 0.001$ compared to saline-treated group (Newman–Keuls post hoc test). Saline—rats i.p. treated with saline; stz-diabetes without neuropathy—rats with streptozotocin-induced diabetes (80 mg/kg, i.p.) without mechanical hypersensitivity as a symptom of neuropathy; stz-diabetes with neuropathy—rats with streptozotocin-induced diabetes with mechanical hypersensitivity as a symptom of neuropathy. Rats which were given STZ i.p. injection, but did not develop signs of diabetes were not tested in paw-pressure test

relationship has been demonstrated between OS, diabetic macro-angiopathy and microangiopathy including peripheral neuropathy [38]. Both micro- and macro-angiopathy and direct neuronal damage caused by chronically elevated intracellular glucose concentrations are implicated in the alterations of the central nervous system in DM. Enhanced intraneuronal glucose oxidation leads to the ROS overproduction within the brain [39] which might overwhelm the antioxidant defense, as supported by the findings of decreased antioxidative enzymes activity in the brain of diabetic rats [7, 8], leading to cell damage. Our results of decreased AC, expressed as $ORAC_{-ROO}^{\bullet}$ and $ORAC_{-OH}^{\bullet}$ (TE, μmol/$g_{fresh\ tissue}$ or μmol/$mg_{proteins}$), in the brain of rats made diabetic by two different BCT drugs are in agreement with that. The regional and FR specificity of the decreased AC in the brain of diabetic rats has been suggested in the present experiments. Both BCT drug (STZ or AL)- and diabetes duration (10 or 21 weeks)-dependency could account for these specificities. Decrement of AC against FR was more spread in AL-induced, longer lasting and metabolically considered more severe diabetes [9], but on the other hand, AC decrement was more severe (50% of C vs. 74–90% of C) in the STZ- than in the AL-induced diabetes, respectively. Interestingly, significantly decreased the $ORAC_{-ROO}^{\bullet}$ was found in the CB of all rats treated with a diabetogenic STZ dose regardless whether the diabetes has been developed or not. Contrary to that, the $ORAC_{-OH}^{\bullet}$ in the CB as well as in the BS did not change in similar conditions. It is important to mention that the AC in the C is region specific with the CB demonstrated the highest AC values against both $OH^{\bullet}$ and $ROO^{\bullet}$ in comparison with the BS and the HPC. Similar uneven distribution of the AC was demonstrated in the rat brain cortex and CB [40]. Possible differences in the brain AC between the two models of diabetes are not likely to be related to the age of animals, as at the time of sacrification they were all ~6 months old and the AC in the brain has not been found significantly declined during aging in the adult and old rats [40]. From the point of view AC is dependent of chemical structure of pure compounds, chemical composition of chemical mixture, chemical composition of complex biological sample and upon FR generator used in assay. Antioxidant capacities measured with different FR generators have different numerical values. Because of this fact it is necessary for estimation of antioxidant status of

Table 4 Antioxidant capacity against hydroxyl ($ORAC_{-OH}^{\bullet}$) and peroxyl ($ORAC_{-ROO}^{\bullet}$) FR in different regions of the rat brain 3 months following the icv treatment of STZ, glucose transport inhibitor 5-thio-D-glucose and their combination, respectively

Region	Treatment	$ORAC_{-OH}^{\bullet}$ (μmol/mg proteins)	$ORAC_{-ROO}^{\bullet}$ (μmol/mg proteins)
CB	C (5)	1,015.5 ± 369.4	3,651.4 ± 1,467.4
	STZ (5)	634.2 ± 185.1*	2,494.4 ± 477.2
	TG (6)	366.4 ± 171.4**	3,108.2 ± 796.9
	TG + STZ (6)	420.8 ± 180.0**	3,772.8 ± 871.1
BS	C (6)	614.7 ± 144.4	2,931.3 ± 1,062.6
	STZ (5)	420.4 ± 174.8*	2,188.4 ± 486.2
	TG (6)	465.7 ± 126.6*	2,216.1 ± 732.9
	TG + STZ (6)	280.7 ± 163.3**	2,169.4 ± 384.7
HPC	C (7)	1,265.7 ± 142.0	1,473.4 ± 145.7
	STZ (7)	1,111.7 ± 103.7	1,383.4 ± 30.8
	TG (7)	868.9 ± 207.4**	1,292.2 ± 84.7*
	TG + STZ (7)	910.9 ± 104.2**	1,384.4 ± 107.8

Data are expressed as mean ± SD; number of animals is given in parenthesis

ORAC oxygen radical absorbance capacity assay, *HPC* hippocampus, *CB* cerebellum, *BS* brain stem, *C* control group, *STZ* streptozotocin intracerebroventricularly (icv) treated group, *TG* 5-thio-D-glucose-icv treated group, *TG + STZ* group with combined TG and STZ icv treatment

*$p < 0.05$

**$p < 0.01$ versus control group by Student's *t*-test

some sample to determine antioxidant score, which is a sum of $ORAC_{-ROO}^{\bullet}$, $ORAC_{-OH}^{\bullet}$, and $ORAC_{-Cu}^{2+}$ (Antioxidant score = $ORAC_{-ROO}^{\bullet}$ + $ORAC_{-OH}^{\bullet}$ + $ORAC_{-Cu}^{2+}$) [24]. For example: ORAC values for green tea were $ORAC_{-ROO}^{\bullet}$ 814, $ORAC_{-OH}^{\bullet}$ 35.8, $ORAC_{-Cu}^{2+}$ –41.9 and antioxidant score was 807.9. A negative $ORAC_{-Cu}^{2+}$ value indicated a Cu^{2+}-initiated prooxidant activity. In this study, AC of hydrophilic brain antioxidants was measured. PDN, a part of long-term diabetic complications known as microangiopathy, has not been investigated in relation to the AC of the brain so far. It seems likely that the PDN does not influence the AC against $ROO^{\bullet}$ and $OH^{\bullet}$ within the investigated brain regions which may suggest that diabetic patients with such a neuropathy would have no additional risk of more extensive OS damage in the brain than those diabetic patients without PDN. However, because of the high individual variations, expressed as a standard deviation, a larger number of samples per a group and more extensive experiments would be needed before drawing a conclusion. Our results demonstrated that STZ-icv treatment although not inducing diabetes is capable of decreasing the $ORAC_{-OH}^{\bullet}$ (for ~13–38%) in the investigated regions of rat brain 3 months following the drug administration while the $ORAC_{-ROO}^{\bullet}$ remained unchanged. This observation is in line with the previous reports of increased OS in the brain of STZ-icv treated rats [19, 20] and strongly supports the hypothesis of STZ-icv rats being a representative experimental model of sAD [16] since the FR generation and OS involvement in the pathophysiology of AD have been recognized [21]. The mechanism of STZ action in the brain has not been explored so far. However, in line with the peripheral toxic effects of these drugs on the insulin producing/secreting cells [9] and altered brain insulin receptor signaling in following the icv drug administration [17], similarity to the peripheral mechanism of action seems likely. STZ betacytotoxicity at the periphery is related to the STZ entering the beta cell through the glucose transporter two (GLUT2) [9], the expression of which has been found also in the rat brain [41]. STZ transport by GLUT2 and consequently diabetes induction is inhibited by glucose transport inhibitor TG [9], but combined TG-icv + STZ-icv treatment has not prevented STZ-icv induced decreased in the $ORAC_{-OH}^{\bullet}$ in none of the three investigated rat brain regions. Interestingly, TG-icv treatment alone also induced decrease in $ORAC_{-OH}^{\bullet}$. These data are in line with the similar cognitive deficits found in TG-icv and STZ-icv treated rats [17] and the fact the combined TG-icv + STZ-icv treatment did not abolished these cognitive deficits [42].

Our results point to the decreased AC in the brain of rats with the experimental DM and rats with the cerebral diabetes representing an experimental model of sAD. The latter model offers a possibility for searching for the new antioxidant drugs in the treatment of neurodegenerative diseases such as sAD.

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