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PROPOFOL PROTECTS RAT ASTROGLIAL CELLS AGAINST TERT-BUTYL HYDROPEROXIDE-INDUCED CYTOTOXICITY; THE EFFECT ON HISTONE AND cAMP-RESPONSE-ELEMENT-BINDING PROTEIN (CREB) SIGNALLING

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Propofol can be potentially beneficial in oxidative stress related malignancies as neurodegenerative diseases and traumatic brain injury but its signalling pathways are poorly understood. In this study effect of propofol on astroglial signalling in oxidative stress was evaluated. Ten days old cultures of rat astroglial cells were treated for 1 hour with t-butyl hydroperoxide (tBHP) to induce oxidative stress following by 1 hour propofol. We measured cytotoxicity, changes in cell growth and apoptosis as well as alterations in expression and acetylation of chromatin core H3 and H4 histone proteins and changes in native and phosphorylated cAMP-response-element-binding protein (CREB). tBHP induced limited cytotoxicity, increased apoptosis, decreased glutamine synthetase and enolase activities, decreased nuclear CREB, CREB-P and histone proteins but unchanged cytosolic CREB and histone acetyltransferase (HDAC) expression. Propofol clearly protected the cells against tBHP-induced toxicity, normalized alterations in cell growth, restored to some extent glial enzyme activities and reduced apoptotic cell numbers. Also, propofol restored H3 but not H4 expression/activation, but was without effect on decreased nuclear CREB expression/activation. These data show that oxidative stress in cultured astroglia significantly affects nuclear CREB and histone proteins and point to the protective role of propofol.

Key words: *astroglia, cAMP-response-element-binding protein, histone acetylation, oxidative stress, propofol*

INTRODUCTION

Propofol (2, 6-diisopropylphenol) is a potent intravenous hypnotic drug widely used in the intensive care units for short-term anaesthesia and for longer-term sedation. Clinical observations indicate that long-term propofol use can be a safe alternative to opiates (1). Propofol receptor-mediated effects involve activation of GABA_A receptors, inhibition of NMDA receptors and alterations in calcium fluxes through slow calcium ion channels (2). Apart from receptor-mediated effects, propofol acts as an antioxidant by scavenging reactive oxygen species and affects intracellular signalling depending on the red-ox state. Propofol was shown to effectively attenuate reperfusion injury in the cerebral cortex (3), kidney (4), cerebral parenchymal arterioles (5) and intestinal mucosa (6). The drug efficiently protected platelets and erythrocytes against oxidative damage (7, 8) and decreased lipid peroxidation in several *in vitro* experiments (9, 10). Thus, propofol can be potentially beneficial in a number of different disorders related to generation of reactive oxygen species, which include brain ischemia/reperfusion injury, tissue inflammation, heart failure, hypertension and arteriosclerosis.

It was shown, that primary cultures of cerebral astrocytes, subject to oxidative stress by incubation with tert-butyl hydroperoxide (tBHP) were protected by delayed administration of anaesthetic concentrations of propofol (9). In this study, the drug

was significantly more potent than α -tocopherol. Glial cells are more resistant than neurons to oxidative stress induced by H₂O₂ or peroxyxynitrite, and are thought to play an important role in brain antioxidant defence (11). In neuron-glia co-culture systems glial cells protect neuronal cells against H₂O₂ toxicity by maintaining sufficient neuronal glutathione levels or directly, by scavenging free radicals (12). Oxidative stress is related to alterations in intracellular signalling, chromatin changes and alterations in gene transcription even if such alterations seem to be less important to cell survival during experimental oxidative stress. Such changes may be however important to the late consequences of stress. The aim of our study was to assess the effects of propofol on signalling pathways related to cell survival, apoptosis and inflammation in oxidative stress, especially on adenosine 3',5'-monophosphate (cAMP) response element-binding protein (CREB) - a histone acetyltransferase (HAT) and on chromatin activation/repression *via* H3 and H4 histone proteins.

MATERIALS AND METHODS

Culture of glial cells

Newborn Wistar rats were obtained from animals bred in the laboratory. Pups were decapitated 24 h after birth, under sterile

conditions, the brain was quickly extracted and rinsed in glial cell culture medium consisting of DMEM/Ham's medium (2/1) supplemented with 20% foetal calf serum (FCS), 2 mM glutamine, 0.001% insulin, 5 mM Hepes, 0.3% glucose and 1% antibiotic-antimycotic solution. The cortex was dissected and the meninges were carefully removed. Then, glial cells were dispersed by gentle aspiration through a sterile needle, 10^6 cells/ml were layered on culture dishes and grown at 37°C in a 5% CO₂ humidified atmosphere. The culture medium was changed on the fourth day after plating to 10% FCS medium and then every three days. Cells were allowed to grow for 10 days and then almost confluent, well differentiated cells consisting mostly of astrocytes were treated with tBHP, propofol or both compounds. The experimental protocol has been approved by the local Ethics Committee of Animal Experimentation according to European guidelines.

Cell treatment

Drug concentration and time of exposure were chosen in initial experiments where astroglial cells were pretreated for 1-6 hours with a 1-1000 μ M tBHP following by 1 hour treatment with 1-1000 μ M propofol. Cells were also treated with corresponding concentrations of propofol or tBHP alone and assayed for cytotoxicity as described. 100 μ M tBHP applied for 1 hour produced significant cytotoxicity and this concentration was used in further experiments in which astroglial cells were switched to antibiotics-free media and then were treated for 1 hour with 100 μ M tBHP. After that, the medium containing tBHP was removed and cells were treated for 1 hour with 5 μ M propofol which is similar to the clinical drug levels during anesthesia. Other cells were treated for 1 hour with 5 μ M propofol alone or treated for 1 hour with 100 μ M tBHP and assayed 1 hour after tBHP removal.

Determination of cell viability

Cell viability and growth rates were assessed in flow cytometry by quantification of the cellular DNA, using propidium iodide (PI) staining in permeabilized cells (13). Briefly, stress-related cellular DNA degradation and changes in cell cycle were assayed on scraped cells originating from the same dissection, stained for 30 minutes with propidium iodide (PI; 50 μ g per ml) in TRIS buffer (100 mM; pH 7.5), containing potassium cyanide (0.1%), NP-40 (0.01%), RNase (40 μ g per ml; Type III-A, 4 KU/ml) and Na₃N (0.1%). The analysis was performed on an aligned Coulter Epics Profile flow cytometer (Coulter, Hialeah, FL, USA) equipped with an argon laser operating at 488 nm. PI fluorescence was measured in 5000 cells/sample with appropriate bandpass filters. DNA histograms were further analysed by DNA quantification software (MultiCycle, Phoenix Flow Systems Inc, San Diego, CA, USA). The cells were quantified by their relative distribution in the damaged-subdiploid G₀/G₁ zone of the DNA fluorescence histograms), diploid (G₀/G₁ zone - pre-DNA synthesis/resting), S-phase (DNA synthesis), and G₂/M (post-DNA-synthesis/mitosis) phases.

Enolase activity

Formation of phosphoenolpyruvate by glial cell-specific enolase (total cytosolic activity) was assayed (14). Reaction was performed at 37°C in 100 mM HEPES buffer, pH 7.0, containing 10 mM MgSO₄ and 7.7 mM KCl and 3 different concentrations of 2-PGE (9-35 mM) in a final volume of 1.0 ml. Changes in absorbance/min were monitored spectrophotometrically at 240 nm. Protein levels were determined using Bio-Rad protein kit (Bio-Rad, Warsaw, Poland).

Glutamine synthetase (GS) activity

GS were determined in the assay mixture of 40 mM imidazole-HCl (pH 7.0), 30 mM L-glutamine, 3 mM MnCl₂, 0.4 mM ADP, 20 mM sodium arsenate, 60 mM NH₂OH and the glial cell homogenate in a final volume of 3 ml. The reaction was stopped after 30 min by adding 1.0 ml of a mixture (1:1:1) of 10% FeCl₃ · x 6H₂O in 0.2 N HCl, 24% TCA and 6 N HCl. The appearance of γ -glutamyl hydroxamate was measured by the increased absorbance at 540 nm (15).

Oxidative stress

Dichlorodihydrofluorescein diacetate (DCFDA) was used to detect the generation of reactive oxygen intermediates in cultured astroglia (16). Cells were stained with 5 μ M DCFDA for 0.5 hour, washed once with PBS, resuspended in PBS and assayed by flow cytometry (Coulter). Green DCF fluorescence was captured on F11 from 2000 cells, shown as histograms of fluorescence distribution and compared.

Annexin V conjugates and apoptosis detection

Annexin V is used to detect apoptosis by targeting for the loss of plasma membrane integrity. A fluorescein isothiocyanate (FITC)-conjugated annexin V (Clontech Labs, Takara BioEurope, Saint-Germain-en-Laye, France) was used to detect apoptotic cells (17). For the analyses, the cells were harvested, washed twice with phosphate-buffered saline pH 7.4, incubated in annexin V-labelling solution (final annexin V concentration - 0.5 μ g/ml), washed and then a second fluorescent dye - PI was added to final concentration 5 μ g/ml. Green and red fluorescences were simultaneously analysed using Coulter flow cytometer calibrated on F11 (annexinV-FITC channel) and F13 (PI channel) using cells stained with annexin V-FITC or PI only. To quantitate early apoptotic cells, annexinV-FITC stained cells that did not fix PI were gated, their green fluorescences were digitized and shown as histograms of fluorescence distribution.

Subcellular fractions and histone isolation

To isolate cytosolic and nuclear fractions, astroglial cells were centrifuged, resuspended in cold hypotonic buffer containing 10 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 50 mM dithiothreitol, 100 mM phenanthroline, 1 mg/ml pepstatin, 100 mM trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane, 100 mM 3,4-dichloroisocoumarin, 10 mM NaF, 100 mM sodium orthovanadate, 25 mM β -glycerophosphate and centrifuged at 14,000 x g for 5 min at 4°C. Cells were lysed in a solution of the same buffer containing 0.2% (v/v) Nonidet P-40 for 10 min on ice and centrifuged at 14,000 x g for 10 min at 4°C. The supernatant was then collected as cytosolic extract. The remaining pellet was resuspended in extraction buffer (20 mM HEPES, pH 7.9, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 25% (v/v) glycerol, 100 mM 3,4-dichloroisocoumarin), incubated for 15 min at 4°C, and centrifuged at 14,000 x g for 10 min at 4°C. The supernatant including soluble nuclear protein was collected as nuclear extract.

Acid extraction of histones was performed in cells treated for 30 min in ice with lysis buffer 10 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT, and 1.5 mM phenylmethylsulfonyl fluoride and hydrochloric acid at a final concentration of 0.2 M and subsequently, lysed cells were centrifuged at 11,000 x g for 10 min at 4°C. Supernatant containing acid-soluble proteins was dialyzed for 1 hour, against 0.1 M acetic acid and then overnight against H₂O and frozen until assayed (18).

Western immunoblotting

Specific proteins were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblotting with antibodies recognizing CREB, Ser 133 phosphorylated CREB (in cytosolic and nuclear extract; Abcam rabbit Abs), histones H3 and H4, and acetylated histones H3 and H4 (AcH3, AcH4 in acid-extracted fractions, Upstate rabbit Abs) and HDAC (in nuclear extract, HDAC2 Santa Cruz rabbit Ab). Histone proteins were separated along with molecular weight markers (Bio-Rad, Hercules, CA, USA) and loading controls in 20% polyacrylamide gels while other proteins were run on 10% SDS gels. Gels were transferred onto 0.45 μ m PVDF membranes (BioRad, Warsaw, Poland). For the negative control study, membranes were treated similarly but without the addition of primary antibody. Species-specific horseradish peroxidase or alkaline phosphatase secondary antibodies were purchased from Santa Cruz or Sigma respectively. Gels were checked for loading using Coomassie staining (histone proteins or nuclear extracts) or B-actin expression (cytosol). Protein bands were quantified using Quantity One software (BioRad, Warsaw, Poland).

Statistical analysis

Statistical analysis was performed with a statistics package - Statistica 6.0 software (Statsoft, Cracow, Poland) using Kolmogorof-Smirnov test to assess data distribution, ANOVA test and Bonferroni post-tests to compare selected pairs of data. Data are shown as mean \pm SD of 5 or 6 assays, P values less than 0.05 were considered significant.

RESULTS

Fig. 1 shows the effect of propofol, tBHP and tBHP-pretreatment following by propofol on cell proliferation and

viability. Propofol was without significant effect on cell growth and no cytotoxicity was detected, while significantly ($p < 0.05$; $n = 6$) increased damaged (subdiploid G0/G1) cell numbers and decreased ($p < 0.05$; $n = 6$) S-phase cell fractions were found in tBHP-treated cells. These changes were absent in cells pretreated with tBHP and then treated with propofol indicating protective effect of the latter drug.

Propofol did not significantly affect culture protein levels and glial marker enzymes - glutamine synthetase and enolase activities in astroglia (Table 1) and induced neither oxidative stress (Fig. 2) nor apoptosis (Fig. 3). Cell treatment with tBHP induced oxidative stress (more than 5 fold increase in DCF fluorescence comparing to untreated cells; $p < 0.01$; $n = 5$; Fig. 2) an increase by about 3 times in apoptotic cell numbers ($p < 0.01$ vs. control; $n = 6$; Fig. 3), a decrease by 41% specific activity of glutamine synthetase ($p < 0.01$ vs. control; $n = 6$; Table 1) and enolase (22% decrease vs. control; $n = 6$; $p < 0.05$; Table 1). Almost all of these changes were normalized by propofol (Table 1, Fig. 2 and Fig. 3) and in cells pretreated with tBHP and treated with propofol, all parameters except for enolase activity were not significantly different from control cells (Table 1, Fig. 3).

Histone acetylation and deacetylation play important roles in the regulation of chromatin structure and gene function (19). Fig. 4 shows representative WB of CREB (which has histone acetyltransferase activity) and activated (Ser 133-phosphorylated) CREB content in nuclear and cytosolic fractions isolated from astroglial cells treated with tBHP or propofol and with tBHP following by propofol. There was not significant change in cytosolic CREB and CREB-P expressions after cell treatment with tBHP or propofol ($p > 0.05$; $n = 5$, but nuclear CREB and CREB-P were decreased respectively by 27% ($p < 0.05$; $n = 6$) and 49% ($p < 0.01$; $n = 6$; Fig. 4). These changes were not affected by propofol. Moreover, significantly decreased (from 3.1 ± 0.41 to 2.3 ± 0.47 ; $p < 0.05$, $n = 6$) nuclear to cytosolic CREB ratio was found after tBHP treatment. This was not altered by propofol and nuclear

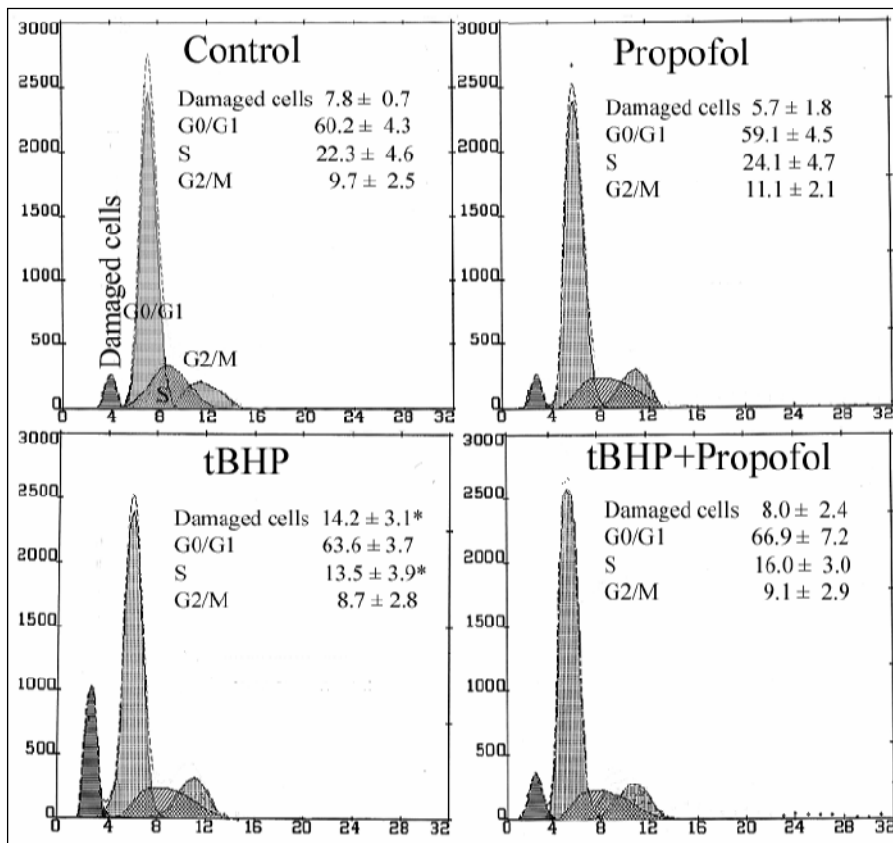


Fig. 1. Representative flow cytometry histograms of propidium iodide fluorescence distributions (MultiCycle transformation) from control astroglial cells, cells treated for 1 hour with 5 μ M propofol, cells treated for 1 hour with 100 μ M tBHP and assayed 1 hour later and cells treated with tBHP as described and then treated for 1 hour with 5 μ M propofol. The cells were quantified by their relative distribution in the damaged-subdiploid G0/G1 zone of the DNA fluorescence histograms, diploid - (G0/G1 zone - pre-DNA synthesis/resting, S-phase - DNA synthesis, and G2/M - post-DNA-synthesis/mitosis phases). Each histogram was derived from analysis of 5000 cells and 6 samples were analysed in each group. *- statistically different from control ($p < 0.05$).

Table 1. Culture protein levels, glutamine synthetase (GS) and enolase activities (mean \pm S.D.) in rat astroglial cells treated for 1 hour with 100 μ M tBHP, 5 μ M propofol or with 100 μ M tBHP following by 5 μ M propofol.

	Culture protein (% of control; n = 8)	GS specific activity (% of control; n = 6)	Enolase specific activity (% of control; n = 6)
Control	100 \pm 9	100 \pm 11	100 \pm 17
Propofol	97 \pm 11	105 \pm 13	108 \pm 16
tBHP	93 \pm 10	59 \pm 16**	78 \pm 18*
tBHP+Propofol	98 \pm 9	87 \pm 17	83 \pm 15*

- statistically different from control ($p < 0.05$; ** $p < 0.01$)

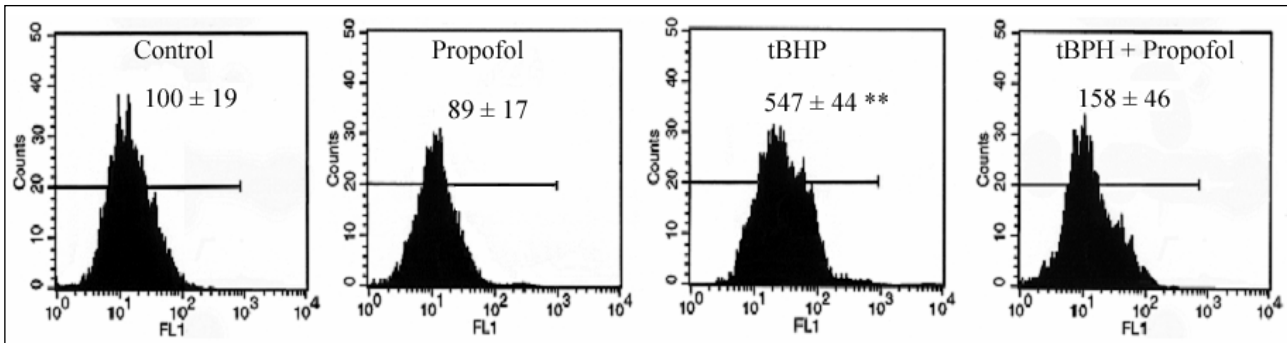


Fig. 2. Representative histograms of dichlorodihydrofluorescein (DCF) fluorescence, reflecting generation of reactive oxygen intermediates in control astroglial cells, cells treated for 1 hour with 5 μ M propofol, cells treated for 1 hour with 100 μ M tBHP and assayed 1 hour later and cells treated with tBHP as described and then treated for 1 hour with 5 μ M propofol. DCF fluorescence was measured in 2000 cells in 5 samples/group. **- statistically different from control ($p < 0.01$).

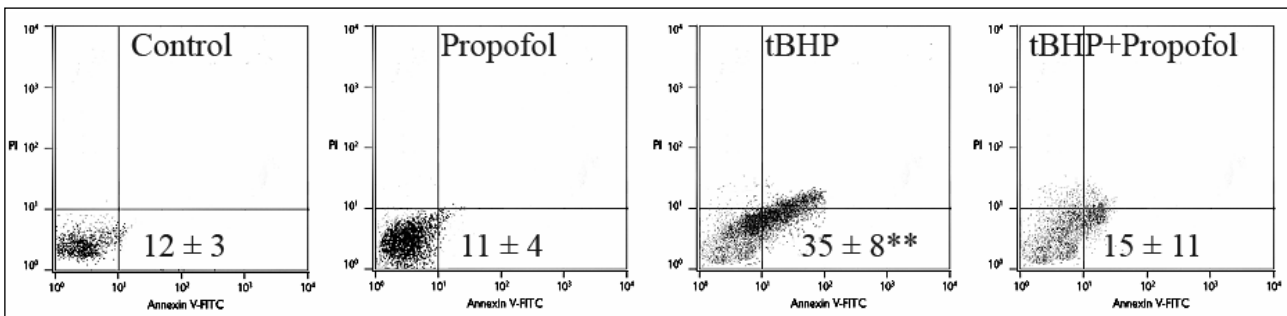


Fig. 3. Representative bivariate cytograms of Annexin V-FITC (green)/propidium iodide (red)-stained control astroglial cells, cells treated for 1 hour with 5 μ M propofol, cells treated for 1 hour with 100 μ M tBHP and assayed 1 hour later and cells treated with tBHP as described and then treated for 1 hour with 5 μ M propofol. Green and red fluorescences were measured in 2000 cells in 5 samples/group. Subpopulation of cells (% of total cell numbers \pm S.D.) which fixed AnnexinV-FITC, but not propidium iodide was gated and considered as apoptotic. **- statistically different from control ($P < 0.01$).

to cytosolic CREB ratio was still decreased (to 1.6 ± 0.22 ; $p < 0.01$; $n = 6$) when tBHP-pretreated cells were treated with propofol.

Fig. 5 show changes in relative expression of H3 and H4 histone proteins, acetylated H3 and H4 proteins (AcH3 and AcH4) and histone deacetylase (HDAC) in untreated astroglial cells, cells treated with tBHP, propofol or with tBHP following by propofol. Propofol was without significant effects on native H3 and H4 or acetylated H3 and H4 and on HDAC expression ($n = 5$; $p > 0.05$) while tBHP induced 60% decrease in H3 ($p < 0.01$; $n = 6$), 27% decrease in AcH3 ($p < 0.05$; $n = 6$), 40% decrease in H4 ($p < 0.01$; $n = 6$) and 31% decrease in AcH4 ($p < 0.05$; $n = 6$) but did not affect HDAC ($p > 0.05$; $n = 5$) levels. tBHP affected mostly unacetylated proteins while decrease in both acetylated histones was relatively lower. In cells treated with tBHP followed by propofol, decrease in H3 and AcH3 was lower ($p < 0.05$; $n = 6$) comparing to tBHP-only treated cells. Similar effect of propofol was not observed in H4 and AcH4 ($p > 0.05$; $n = 5$). Both compounds did not affect HDAC expression ($p > 0.05$; $n = 5$).

DISCUSSION

Propofol is widely used for the induction and maintenance of general anaesthesia or sedation. The drug acts classically *via* neuronal the GABA_A channel (2). Apart from receptor-mediated effects, propofol can alter cellular red-ox balance due to its strong antioxidative potential (20), decrease blood pressure (21) and metabolism (22). Thus, propofol can be potentially useful in the treatment of brain diseases related to oxidative stress. Glial cells play important roles in brain defense against oxidative stress which is involved in both neurodegenerative diseases (23) and traumatic brain injury (24). It was shown, that in cultured astrocytes propofol attenuated peroxynitrite-mediated apoptosis with involved nuclear factor kappaB (NF- κ B) (25), increased HO-1 expression (26), affected glial Ca²⁺ channels (27), restored intracellular ascorbate (28) and was definitely more potent than alpha-tocopherol in attenuating decreased glutamate uptake induced by tBHP (9). Clinically important levels of propofol

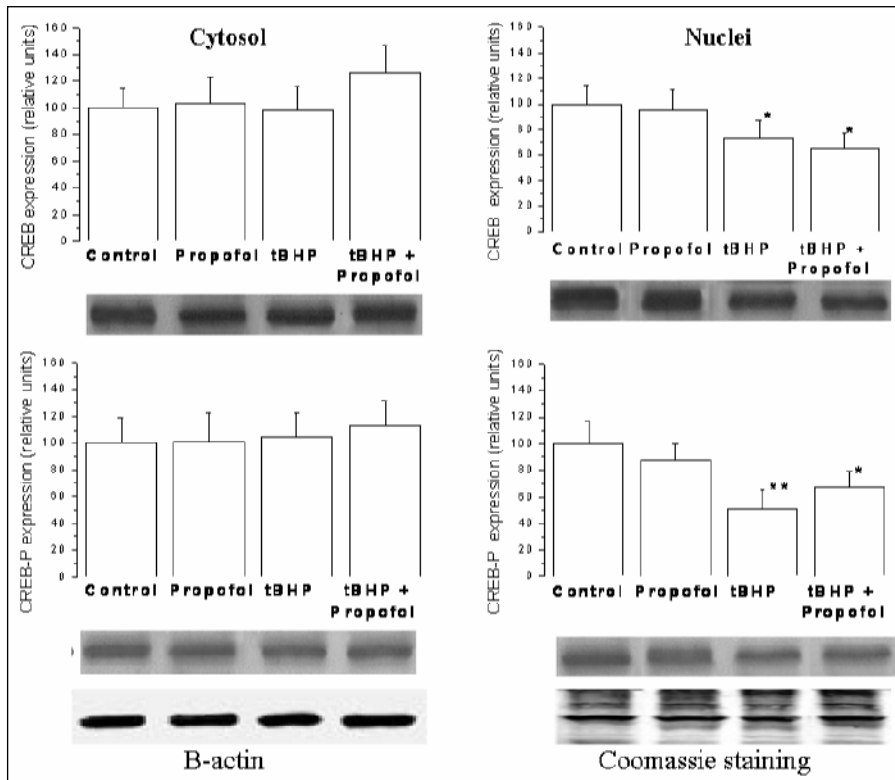


Fig. 4. Representative Western-blots of CREB and activated (Ser133-phosphorylated) CREB (CREB-P), in cytosol and nuclei of control astroglial cells, cells treated for 1 hour with 5 μ M propofol, cells treated for 1 hour with 100 μ M tBHP and assayed 1 hour later and cells treated with tBHP as described and then treated for 1 hour with 5 μ M propofol. Corresponding quantification bars (mean expression \pm S.D. of 6 assays in each group) and representative loading controls are also shown. Statistically different from control (*- P<0.05; ** - P<0.01).

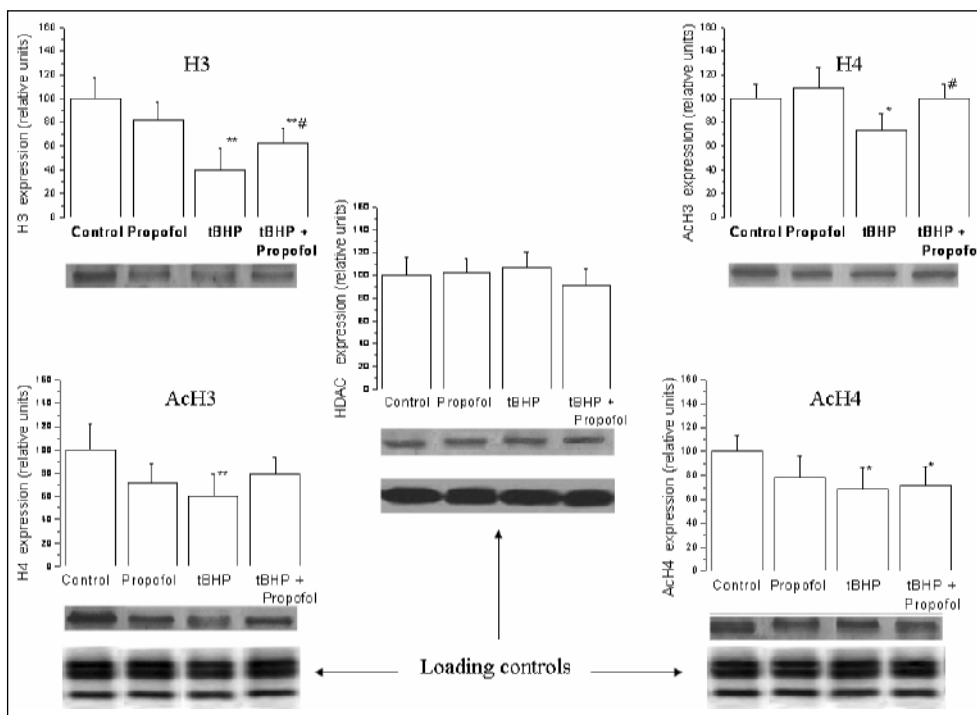


Fig. 5. Representative Western-blots, corresponding quantification bars (mean expression \pm S.D. of 5 or 6 assays) and loading controls of H3, acetylated H3 (AcH3), H4, acetylated H4 (AcH4) histone proteins and histone acetyltransferase (HDAC) in control astroglial cells, cells treated for 1 hour with 5 μ M propofol, cells treated for 1 hour with 100 μ M tBHP and assayed 1 hour later and cells treated with tBHP as described and then treated for 1 hour with 5 μ M propofol. Equal gel loading was verified using Coomassie staining (gels with histone proteins) or β -actin expression (HDAC). Statistically different from control (*- P<0.05; ** - P<0.01) or from tBHP-treated cells (# - P<0.05).

evidently neutralize the effects of exogenous oxidative stress in cultured glial cells and such protection seem to be independent of GABA_A receptor.

DNA microarray data have shown that propofol stimulates antioxidant pathways and increases chaperone proteins but also induces proinflammatory and prothrombotic genes (29). Both mechanisms appear to be closely correlated at the level of nucleosome remodelling pathways, which are important regulatory mechanisms for the transcriptional activation of pro- and antiinflammatory genes and for reparation of damaged DNA (30, 31).

In our experiments oxidative stress was induced in rat astroglia by tBHP and then cells were treated with propofol. Time of treatment and tBHP concentration were chosen to induce restricted cytotoxicity, thus it did not significantly affect total culture protein levels but altered cellular DNA, glial-specific enzymes and induced apoptosis. Clinically relevant concentration of propofol clearly protected the cells against tBHP-induced cytotoxicity. The drug normalized alterations in cell growth, restored to some extent glial enzyme activities and also protected the cells against apoptosis. Propofol also partly normalized oxidative stress. Similar protective proprieties of propofol were

already described in cultured glial cells, however, their mechanism is not clear (9, 10). Additional studies with alternative antioxidants are necessary to better understand such mechanisms but it seems that important role in drug signalling is played by altered cellular red-ox state due to scavenging properties of propofol. Such changes are integrated at the nuclear level resulting in chromatin remodelling, they play important roles in oxidative and inflammatory stress-related diseases such as cancer, viral infections, autoimmune diseases, Alzheimer disease, asthma and COPD and may be targeted by therapy (31, 32, 33). Among posttranslational modifications of proteins altered histone acetylation is now recognized as a major factor affecting chromatin signal transduction. Generally, acetylation of histone proteins by histone acetyltransferases (HAT) activates transcription machinery, while deacetylation, mediated by HDAC down-regulates gene activation (34). CBP (CREB (cAMP-response-element-binding protein)-binding protein) contains HAT domains and serves as co-activator of gene expression, but there is only scarce data on this molecule in the context of propofol signalling. Recent report showed, that clinically relevant concentrations of the drug reduce NMDA receptor-mediated ERK phosphorylation in neurons, resulting in inhibition of CREB and decreased CREB-dependent reporter gene (c-Fos) expression (35). In contrast, in cerebral ischemia neuronal CREB and CREB-P are elevated (36). In our model oxidative stress decreased nuclear but not cytosolic CREB and CREB-P. Thus, oxidative stress appears to decrease nuclear CREB-mediated signalling. In neuronal and non neuronal cells CREB activation may lead to expression of genes encoding neuroprotective molecules, such as the antiapoptotic protein Bcl-2, contribute to survival of cells after ischemic insult (36) and participate in cell response to antiinflammatory signaling (37, 38). However, CREB may also affect proinflammatory molecules like NF- κ B (39, 40, 41). In our experiments propofol neither altered nor normalized nuclear CREB or activated CREB expression, however the corresponding response in neurons may be different due to high neuronal density of GABA_A receptors. Considering protective effects of propofol on glial cell survival and apoptosis, its inability to normalize decreased CREB expression in cells subjected to oxidative stress might indicate that CREB-related signalling was less important for cell resistance. On the other hand, propofol was also found to significantly decrease oxidative stress, and possibly reverse some late changes, although it is still possible that this neutralization is stronger in cytosol than in cell nuclei.

Propofol itself was without effect on native and acetylated H3 and H4 and on HDAC expression while tBHP induced significant decrease in H3, AcH3, H4, AcH4. tBHP affected mostly unacetylated protein levels because relative decrease in both acetylated histone proteins was lower. The drug normalized to some extent alterations induced by tBHP in H3 but not in H4 and did not affect HDAC expression.

High levels of cellular oxidative stress decrease gene transcription and this decrease correlates with decreased histone H3 and H4 acetylation (42), which is regulated in part by cAMP-dependent CREB. This reaction is very important in inflammatory diseases because hyperacetylated histones are associated with enhanced inflammatory gene expression (31, 43). Thus, HDAC enzymes become potential therapeutic targets in several diseases like ischemia, neurodegeneration and cancer (19). It was shown, that clinically relevant concentrations of propofol increased the V_{max} without affecting the K_m for phosphorylation of PKC-mediated phosphorylation of histone H1 (44), but there is no data on H3, H4 and on their acetylation status. It was shown, that propofol increased HO-1 expression, decreased DNA damage and caspase-3 activation in cultured astroglia, while inhibition of NF- κ B abolished propofol-mediated effects (28). Our data show, that oxidative stress affect

chromatin status in cultured astroglia and points to some protective role of propofol, but also suggest that H3 and H4 histone homeostasis can be independently regulated.

Although, information obtained from cultured astroglial cells should be taken with caution when extrapolated to *in vivo* situation, our results actually show that, propofol may modify signal transduction pathways to elicit beneficial effect in patients undergoing anesthesia who are at risk of increased organ injury from tissue oxidants.

Acknowledgements: This work was supported by a Grant 366902 from the Medical University of Białystok.

Conflict of interests: None declared.

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Received: January 5, 2009

Accepted: November 6, 2009

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