Research report

Effect of propofol on oxidative stress in an in vitro model of anoxia-reoxygenation in the rat brain

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Abstract

Propofol, an intravenous anaesthetic, is similar in chemical structure to the active nucleus of antioxidant substances such as α-tocopherol (vitamin E). The present study was designed to test whether propofol had antioxidant effects in an in vitro model of anoxia-reoxygenation in slices of rat brain. We used seven experimental groups: (1) control oxygenated tissue; (2) tissue subjected to anoxia for 20 min and reoxygenation for 3 h; and tissues processed as described and incubated with (3) Intralipid (commercial solvent for propofol), or propofol at a concentration of (4) 10 μmol/l, (5) 50 μmol/l, (6) 150 μmol/l or (7) 300 μmol/l. The production of lipid peroxides was quantified as thiobarbituric acid reactive substances (TBARS); tissue glutathione production and the activities of glutathione peroxidase (GSHpx), glutathione reductase (GSSGrd) and glutathione transferase (GSHtf) were also measured. Reoxygenation led to tissue oxidative stress, which was curtailed by propofol. The anaesthetic led to a 47% reduction in TBARS, a 165% increase in the reperfusion-inhibiting glutathione content, a 47% decrease in GSHpx activity, and an 87% increase in GSHtf activity. Intralipid had no effect on any of the parameters studied here. We conclude that propofol has a clear antioxidant effect in rat brain tissue subjected to anoxia-reoxygenation.

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Keywords: Propofol; Ischemia; Reperfusion; Oxidative stress; Glutathione; Lipid peroxidation

1. Introduction

The production of free radicals derived from oxygen, which leads to the production of lipid peroxides in cell membranes [2,5,7,23], is one of the major mechanisms of tissue damage in processes of ischemia-reperfusion. The increase in oxidative damage is usually accompanied by a decrease in antioxidant defences, especially impairment of the glutathione system [2,5,7,23].

Several methods exist to analyse the damage produced by ischemia-reperfusion in brain tissue in terms of free radical production, and to test whether antioxidant drugs protect the tissue from the effects of free radicals [15]. When vivo models are used, vascular factors can have a decisive influence on the final effect of the drug, such that any protective effect cannot be attributed with complete confidence to a direct action of the drug in the brain tissue or to its effect on blood circulation. To overcome this obstacle, in vitro models of anoxia-reoxygenation in slices of brain tissue attempt to simulate processes of ischemia-reperfusion in isolation from vascular factors [8].

The intravenous anaesthetic propofol is similar in chemical structure to the active nucleus of α-tocopherol and butylhydroxytoluene, two substances with antioxidant properties. The antioxidant effect is the result of inhibition of lipid peroxidation by the formation of relatively low reactive free radicals that disrupt the chain of formation of other free radicals potentially able to cause greater damage to cell membranes [1,12,26].

These effects suggested that propofol might interfere with alterations in oxidative status in brain tissue during ischemia and reperfusion. The present study was designed to test whether propofol modifies tissue oxidative balance in an in vitro model of anoxia-reoxygenation in the rat brain.

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2. Material and methods

2.1. Animals and experimental model

This in vitro study was done with brain tissue from male Wistar rats (body weight 300–350 g). All animals were housed under standard conditions with food and water available ad libitum. The study was performed in compliance with international guidelines for the care and handling of laboratory animals.

The rats were killed by decapitation and the whole brain was removed immediately. The cerebellum and brain stem were discarded and the remaining tissue was cut transversally into 2-mm slices (298 ± 4.4 mg) with a vibrotome (Capdem Instruments, USA) according to a previously described technique [8] with slight modification. The slices were placed in buffer (composition in M:0.1 NaCl, 5 × 10⁻⁴ KCl, 2.4 × 10⁻² NaHCO₃, 5.5 × 10⁻⁴ KH₂PO₄, 5 × 10⁻⁶ CaCl₂, 2 × 10⁻⁷ MgSO₄, 9.8 × 10⁻³ glucose, pH 7.4) perfused with a mixture of 95% O₂ and 5% CO₂. After 30 min to reach equilibrium the slices were placed in fresh buffer of the same composition except that the concentration of CaCl₂ was 3 × 10⁻³ M, that of MgSO₄ was 1 × 10⁻⁶ M, and no glucose was included. This solution was perfused with a mixture of 95% N₂ and 5% CO₂ (reoxygenation).

One brain slice was analysed for each of the following conditions: (1) after 30 min of incubation and before N₂ perfusion, (2) after 20 min of perfusion with N₂, (3) 1 h after reoxygenation, (4) 2 h after reoxygenation, (5) 3 h after reoxygenation. For all studies, the tissues were quickly frozen in liquid nitrogen and stored at −80°C until the day of the experiment, which was done within 7 days of freezing.

Seven different groups of ten rats each were used: (1) brain tissue perfused with 95% O₂ and 5% CO₂ for the entire experiment (control), (2) anoxiareoxygenation, (3) anoxiareoxygenation with 10 μmol/l propofol (Zeneca Farma, Madrid, Spain) in the incubation medium, (4) with 50 μmol/l propofol, (5) with 150 μmol/l propofol, (6) with 300 μmol/l propofol, and (7) with 10% Intralipid (KaviPfimmer, Barcelona, Spain), the commercial solvent used with propofol.

2.2. Lipid peroxidation

To quantify lipid peroxidation, we measured thiobarbituric acid reactive substances (TBARS) under basal conditions and after induction with ferrous salt [10]. Cell membrane-enriched fractions of the tissue samples were obtained as described by Bossman and Hemsworth [4]. Briefly, the tissue was diluted (1:10 wt./vol) in a buffer consisting of 0.1 M NaCl, 5 × 10⁻⁴ M KCl, 3.1 × 10⁻³ M CaCl₂, 1 × 10⁻³ M MgSO₄, 4.9 × 10⁻³ M glucose, 2.4 × 10⁻² M Na₂CO₃, 5.5 × 10⁻⁴ M PO₄H₂K and 0.32 M sucrose. The sample was homogenised and centrifuged at 10 000 × g for 15 min at 4°C, and the supernatant was collected and centrifuged again at 12 000 × g for 20 min at 4°C. The resulting pellet was resuspended in the same buffer without sucrose at a proportion appropriate for the determination of lipid peroxide production.

Lipid peroxides were determined by dividing the tissue into 850-μl aliquots and adding 100 μl dilution buffer per tube (basal lipid peroxidation) or 100 μl of ferrous sulfate and ascorbic acid (FeAs) at increasing concentrations (induced lipid peroxidation). The tubes were shaken and incubated at 37°C for 45 min, then 500 μl of 0.5% thiobarbituric acid in 20% trichloroacetic acid was added. The samples were shaken and incubated at 100°C for 15 min, then centrifuged at 2000 × g for 15 min at 4°C. Absorbance of the resulting supernatant was determined spectrophotometrically at 532 nm (Perkin Elmer C-532001 spectrophotometer, USA). Blank samples were prepared in an identical manner except that they were incubated at 4°C. The results were expressed as μmol TBARS/mg protein; the latter was determined with the method of Lowry et al. [24].

2.3. Glutathione levels

Total glutathione was measured spectrofluorometrically according to the technique described by Hissin and Hill [22]. Briefly, 200 mg of brain tissue was homogenised in 0.1 M sodium phosphate buffer (pH 8.0) with 25% phosphoric acid at a proportion of 1:20, then centrifuged at 13 000 × g for 15 min at 4°C to obtain the supernatant. Duplicate cuvettes were prepared for spectrofluorometry with the following components: 1.8 ml sodium phosphate buffer, 100 μl supernatant for each sample, and 100 μl o-phthaldehyde. The cuvettes were shaken and incubated for 15 min at 4°C, then read at an excitation wavelength of 350 nm and an emission wavelength of 440 nm. The results were compared with those of a standard curve for commercial glutathione that was processed in an identical manner, and were expressed as μmol of glutathione/g of tissue.

To determine the proportions of oxidised and reduced glutathione, we incubated 200 μl of supernatant from each sample with 8 μl 4-vinylpyridine for 1 h at room temperature, then proceeded as described above for total glutathione. The resulting figure represented oxidised glutathione (GSSG); reduced glutathione (GSH) was considered the difference between total glutathione and GSSG.

2.4. Activities of glutathione-related enzymes

Enzyme activities were measured as spectrophotometric kinetics. Tissue samples weighing 300–400 mg were diluted in 4 ml 0.1 M potassium phosphate buffer (pH 7.0) with 1 ml 25% phosphoric acid. The samples were ho-
Table 1  
Mean values of lipid peroxidation and glutathione status in anoxia-hyperoxia control \(O_2/N_2\) control \((n = 10)\) and oxygen control \(O_2\) control \((n = 10)\) rat brain slices

<table>
<thead>
<tr>
<th></th>
<th>(O_2) control</th>
<th>(O_2/N_2) control</th>
<th>(1\ h\ O_2)</th>
<th>(2\ h\ O_2)</th>
<th>(3\ h\ O_2)</th>
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<tbody>
<tr>
<td><strong>Non-induced TBARS (nmol/mg protein)</strong></td>
<td></td>
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<tr>
<td>Pre-(N_2)</td>
<td>8.2 ± 0.6</td>
<td>8.6 ± 0.2</td>
<td>7.7 ± 0.3</td>
<td>7.4 ± 0.8</td>
<td>7.7 ± 0.1</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>8.5 ± 0.7</td>
<td>7.8 ± 0.8</td>
<td>10.1 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.8 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Induced TBARS (nmol/mg protein)</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Pre-(N_2)</td>
<td>131 ± 8.7</td>
<td>130 ± 7.7</td>
<td>134 ± 5.5</td>
<td>140 ± 4.1</td>
<td>140 ± 4.3</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>132 ± 11.3</td>
<td>122 ± 12.9</td>
<td>143 ± 15.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>165 ± 15.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168 ± 12.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>GSH + GSSG ((\mu)mol/g tissue)</strong></td>
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<tr>
<td>Pre-(N_2)</td>
<td>5.4 ± 0.5</td>
<td>5.3 ± 0.7</td>
<td>4.5 ± 0.6</td>
<td>4.4 ± 0.6</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>5.7 ± 0.4</td>
<td>5.4 ± 0.5</td>
<td>4.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>GSH ((\mu)mol/g tissue)</strong></td>
<td></td>
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<tr>
<td>Pre-(N_2)</td>
<td>4.9 ± 0.3</td>
<td>4.8 ± 0.2</td>
<td>4.0 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>5.1 ± 0.6</td>
<td>4.8 ± 0.5</td>
<td>4.2 ± 0.4</td>
<td>2.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>%GSSG vs. total glutathione</strong></td>
<td></td>
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<tr>
<td>Pre-(N_2)</td>
<td>9.8 ± 0.6</td>
<td>10.0 ± 0.2</td>
<td>10.3 ± 1.1</td>
<td>14.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>10.7 ± 1.2</td>
<td>10.1 ± 0.4</td>
<td>11.1 ± 0.4</td>
<td>11.3 ± 0.8</td>
<td>11.7 ± 0.6</td>
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<tr>
<td><strong>GSHpx activity ((\mu)mol/min)</strong></td>
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<td></td>
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<tr>
<td>Pre-(N_2)</td>
<td>5.4 ± 0.7</td>
<td>5.5 ± 0.7</td>
<td>5.3 ± 0.5</td>
<td>4.9 ± 0.5</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>5.7 ± 0.6</td>
<td>5.6 ± 0.8</td>
<td>8.0 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.1 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.8 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>GSSGrd activity ((\mu)mol/min)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Pre-(N_2)</td>
<td>1.9 ± 0.08</td>
<td>1.8 ± 0.05</td>
<td>1.8 ± 0.09</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.09</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td><strong>GSHtf activity ((\mu)mol/min)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pre-(N_2)</td>
<td>2.6 ± 0.4</td>
<td>2.9 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>Post-(N_2)</td>
<td>2.8 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>1.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> <sup>p</sup><sub>0.05</sub> vs. \(O_2\) control.  
<sup>b</sup> <sup>p</sup><sub>0.05</sub> vs. pre-\(N_2\) value.
Table 2
Effects of propofol in rat brain slices under oxygenation conditions (n = 10 rats per group)

<table>
<thead>
<tr>
<th></th>
<th>Intralipid</th>
<th>Propofol 10 μmol/l</th>
<th>Propofol 50 μmol/l</th>
<th>Propofol 150 μmol/l</th>
<th>Propofol 300 μmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-induced TBARS (nmol/mg protein)</td>
<td>8.8 ± 0.6 (107)</td>
<td>6.9 ± 0.1* (84.1)</td>
<td>5.6 ± 0.4* (68.3)</td>
<td>4.9 ± 0.6* (59.7)</td>
<td>3.0 ± 0.3* (36.5)</td>
</tr>
<tr>
<td>Induced TBARS (nmol/mg protein)</td>
<td>135 ± 6.5 (103)</td>
<td>123 ± 8.8 (93.9)</td>
<td>111 ± 8.2* (84.7)</td>
<td>83.4 ± 9.3* (63.6)</td>
<td>68.3 ± 7.6* (52.1)</td>
</tr>
<tr>
<td>GSH + GSSG (μmol/g tissue)</td>
<td>5.5 ± 0.5 (101)</td>
<td>5.5 ± 0.6 (102)</td>
<td>6.6 ± 0.8* (122)</td>
<td>6.7 ± 0.6* (124)</td>
<td>8.4 ± 0.8* (155)</td>
</tr>
<tr>
<td>GSH (μmol/g tissue)</td>
<td>4.9 ± 0.5 (100)</td>
<td>4.9 ± 0.4 (100)</td>
<td>5.6 ± 0.4 (114)</td>
<td>5.8 ± 0.4* (119)</td>
<td>7.0 ± 0.5* (144)</td>
</tr>
<tr>
<td>GSSG vs. total glutathione</td>
<td>10.1 ± 0.5 (94.3)</td>
<td>10.5 ± 0.7 (98.1)</td>
<td>12.5 ± 0.8 (116)</td>
<td>13.7 ± 0.8* (128)</td>
<td>15.9 ± 0.8* (148)</td>
</tr>
<tr>
<td>%GSSG vs. total glutathione</td>
<td>5.5 ± 0.4 (102)</td>
<td>6.0 ± 0.3 (111)</td>
<td>6.9 ± 0.4* (127)</td>
<td>7.8 ± 0.3* (144)</td>
<td>8.9 ± 0.3* (165)</td>
</tr>
<tr>
<td>%GSHpx activity (μmol/min)</td>
<td>5.1 ± 0.1 (101)</td>
<td>2.1 ± 0.09 (110)</td>
<td>2.5 ± 0.3 (131)</td>
<td>2.5 ± 0.3 (131)</td>
<td>2.8 ± 0.3* (147)</td>
</tr>
<tr>
<td>%GSSGrd activity (μmol/min)</td>
<td>2.4 ± 0.2 (92.3)</td>
<td>2.3 ± 0.08 (88.5)</td>
<td>2.9 ± 0.2 (111)</td>
<td>3.4 ± 0.09* (130)</td>
<td>4.6 ± 0.2* (177)</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. O2 control value (Table 1).
Values in parentheses are the percentage of the oxygenated control value (Table 1).
mogenised and centrifuged at 13 000×g for 15 min at 4°C, and proteins were analysed in the supernatant. Enzyme activities were determined as described in Sections 2.4.1, 2.4.2 and 2.4.3.

2.4.1. Glutathione peroxidase activity

(GSHpx) was measured with the method of Flohe and Gunzel [14]. Briefly, to a volume of each supernatant equivalent to 25 μg protein, we added an appropriate volume of 0.1 M potassium phosphate buffer in order to obtain a final volume of 880 μl, 53 μl reduced glutathione (GSH) and 100 μl NADPH. The microcuvette was shaken by inversion and incubated at 37°C for 3 min. Then 100 μl tert-butylhydroperoxide was added, and the signal was read at 340 nm for 5 min, recording the decrease in absorbance every 30 s.

2.4.2. Glutathione reductase activity

(GSSGrd) was determined with the method of Flohe and Gunzel [14]. The vols. of sample and buffer were the same as for GSHpx assays. After 100 μl NADPH was added, the microcuvette was shaken by inversion and incubated as described above. Then 100 μl GSSGrd was added and the sample was shaken again and read spectrophotometrically at 340 nm, recording the decrease in absorbance every 30 s.

2.4.3. Glutathione transferase activity

(GSHtf) was determined with the method of Warholm et al. [32]. Vols. of sample and buffer as in the above two fractions were mixed with 100 μl GSH by inversion and incubated for 3 min at 37°C. Then 50 μl 1-chloro 2,4-dinitrobenzene was added and the sample was shaken and read at 340 nm as for GSHpx and GSSGrd activity.

The results of the GSHpx and GSSGrd assays were expressed as units per minute, using a molar extinction coefficient of 6.22 cm²/μmol for NADPH, and a correction coefficient of 0.1042 for GSHtf.

2.5. Statistical methods

The data in the text, tables and figures are expressed as the mean ± S.E.M. from ten experiments in samples from different animals. All statistical analyses were done with the Social Program for Statistical Sciences (SPSS v. 6.0). One-way ANOVA followed by Bonferroni transformation was used, and differences were considered significant when p < 0.05.

3. Results

With the in vitro model of anoxia-reoxygenation used here, we found changes in all components of oxidative status in rat brain slices (Table 1). A 20-min period of anoxia led to no significant changes in any of the parameters. Reoxygenation after anoxia increased the production of non-induced TBARS (maximum increase 160±10.2% after 3 h in comparison to the preanoxia value), and of TBARS induced with ferrous salt (mean increase 120±8.0% after 3 h). Glutathione production decreased 3 h after reoxygenation by 56.2±4.2% in comparison with the preanoxia value, whereas the percentage of oxidised glutathione increased by a maximum of 166±11.6% 3 h after reoxygenation. With regard to enzyme activities related with the glutathione system, an increase in GSHpx of

![Fig. 1. Percentages of non-induced (□) and induced TBARS (■) with respect to preanoxic values, after 3 h of reoxygenation in non-anoxic controls (O₂), and in anoxic—hyperoxic slices without drugs (O₂/N₂) or treated with Intralipid or propofol. Each bar is mean ± S.E.M. of ten independent experiments. *p < 0.05 vs. the O₂ group. +p < 0.05 vs. the O₂/N₂ group.](image)
Fig. 2. Percentages of total glutathione content (□) and percentage of glutathione in the oxidised form (GSSG) (●) with respect to preanoxic values, after 3 h of reoxygenation in non-anoxic controls (O₂), and in anoxic–hyperoxic slices without drugs (O₂/N₂) or treated with Intralipid or propofol. Each bar is the mean ± S.E.M. of ten independent experiments. * p < 0.05 vs. the O₂ group, +p < 0.05 vs. the O₂/N₂ group.

189 ± 15% in comparison with the preanoxia value was accompanied by decreases in GSSGrd (74.0 ± 6.1%) and GSHtf (50.6 ± 4.8%) 3 h after reoxygenation.

Table 2 summarises the effects of Intralipid and propofol on oxidative status in brain rat slices before perfusion with N₂. Intralipid did not significantly modify any of the parameters measured in this study. Propofol led to a concentration-dependent reduction in lipid peroxides both under basal conditions and after induction with ferrous salt. Total glutathione was increased in a concentration-dependent manner, whereas the percentage of oxidised glutathione was unchanged. Propofol significantly increased the activity of GSHpx and GSHtf, but had no effect on GSSGrd activity.

About 3 h after reoxygenation, Intralipid did not significantly modify any of the oxidation parameters (Figs. 1–3).

Fig. 3. Percentages of GSHpx (□), GSSGrd (●) and GSHtf (●) activities with respect to preanoxic values, after 3 h of reoxygenation in non-anoxic controls (O₂), and in anoxic–hyperoxic slices without drugs (O₂/N₂) or treated with Intralipid or propofol. Each bar is the mean ± S.E.M. of ten independent experiments. * p < 0.05 vs. the O₂ group, +p < 0.05 vs. the O₂/N₂ group.
Propofol diminished, in a concentration-dependent manner, the increase in lipid peroxide production in the control group: non-induced TBARS production was reduced by a maximum of 79% in comparison with slices not treated with propofol 3 h after reoxygenation, and induced TBARS production was reduced by 47% (Fig. 1).

The decrease in glutathione production caused by anoxia-reoxygenation was reversed by propofol. Total glutathione was 165% in comparison with the value found 3 h after reoxygenation in the untreated control group, and did not differ significantly in comparison with the group treated after reoxygenation in the untreated control group, and did not receive propofol. The activity of GSHpx, which was increased in the absence of the drug, was gradually decreased by propofol treatment (maximum reduction of 31%, 3 h after reoxygenation in comparison with the control group that did not receive propofol).

The presence of propofol in the incubation medium decreased oxidising factors and preventing the decrease in the antioxidant defences in brain tissue. The ability of the anaesthetic to inhibit the increase in lipid peroxide production may stem from an antioxidant effect of propofol itself, or of its commercial solvent Intralipid. However, we ruled out the latter possibility, as Intralipid alone had no significant effect on any of the parameters in oxygenated tissues (Table 2) or in tissues subjected to experimental anoxia-reoxygenation (Table 2).

We found that propofol inhibited lipid peroxidation in tissues before anoxia (Table 2) and after anoxia-reoxygenation (Fig. 1). These results are concordant with earlier findings that the anaesthetic reduced peroxidation in a dose-dependent manner in several different rat tissues [7–10]. Previous studies postulated that propofol was able to bind free radicals and thus prevent them from interacting with other molecules. Studies in a variety of biological and chemical media [1,13,26,27] have also reported an antioxidant effect for this anaesthetic, and have suggested that it might act like two chemically similar molecules, butylhydroxytoluene and α-tocopherol. These molecules bind to the cell membrane or to phospholipids to form free radicals that are much less reactive than those that would form otherwise. In a rat model of ischemia-reperfusion, α-tocopherol has been shown to protect brain tissue in direct relation with its antioxidant effect [18,31]; propofol may act through a similar mechanism. In the lipid peroxidation experiments, we used ferrous salts as inducer, so propofol could act as an iron chelating agent, however the studies of Green et al. [16] and Hans et al. [17] demonstrated this antiperoxidative effect of propofol, in the same range of concentrations, using other chemical lipid peroxidation inducers different from ferrous salts. In addition, propofol interfered with human platelet aggregation in vitro [9], an effect that may also help limit cerebral ischemia; further research will be needed to confirm the relationship between these effects. Some synthetic drugs able to inhibit lipid peroxidation have also been shown to reduce brain injury in models of ischemia-reperfusion [3,11].

Another important aspect to consider in tissular oxidative damage is the effectiveness of the glutathione antioxidant defence system. The model of anoxia-reoxygenation used in the present study modified some components of oxidative status in rat brain slices. We found that damaging oxidative reactions tended to increase, while the cell’s antioxidant defences (represented here by the glutathione system) were inhibited. These results are consistent with earlier findings [2,5,7,23] that pointed toward an important role for the production of oxygen-derived free radicals, or impairment of the mechanisms that prevented the production of free radicals, in the pathophysiology of brain tissue damage during processes of ischemia and reperfusion. In previous experiments (data not shown), we carried out a time course study using different times of ischemia; the peroxidative damage were time-related, but the effect of propofol was proportionally the same, for that reason we used a short ischemic period, in order to shorten the total time of the experiment.

In our model, tissue reoxygenation led to biochemical changes (increased TBARS production) and evidence that a tissular defence mechanism was triggered (increased GSHpx activity). These changes correlated with an increase in the percentage of oxidised glutathione. However, the concomitant increase in lipid peroxidation suggests that this defence mechanism was neither sufficient nor entirely effective.

The presence of propofol in the incubation medium brought about a partial re-equilibration of oxidative status, decreasing oxidising factors and preventing the decrease in the antioxidant defences in brain tissue. The ability of the anaesthetic to inhibit the increase in lipid peroxide production may stem from an antioxidant effect of propofol itself, or of its commercial solvent Intralipid. However, we ruled out the latter possibility, as Intralipid alone had no significant effect on any of the parameters in oxygenated tissues (Table 2) or in tissues subjected to experimental anoxia-reoxygenation (Figs. 1–3).
approach comparable with ours, using propofol, although one study reported a relationship between the prevention of ischemic brain damage and increased glutathione production in the brain [12], a finding compatible with our results. Another study by Aarts et al. [1] found that propofol and GSH reduced lipid peroxidation in the rat liver in a synergistic manner. The fact that the same drug inhibited lipid peroxidation while stimulating the glutathione system, measured as a prevention of its inhibition in our experimental model, is evidence in support of an antioxidant action, as propofol not only affected aggressive oxidising mechanisms, but also influenced endogenous antioxidative defences.

It has been demonstrated that mitochondrial function is altered in ischemic-reperfusion models, mainly by a dysfunction of its respiratory activity, caused by oxygen radicals [19], intracellular pH [20], calcium homeostasis [21], and degradation of mitochondrial phospholipids [28]. Mitochondria have high content of glutathione, and propofol inhibits lipid peroxidation in isolated rat mitochondria [13], which supports a possible effect of propofol in this subcellular level.

Another antioxidant system against free radicals is superoxide dismutase (SOD). Investigations in animal models of cerebral ischemia suggest a particular role of SOD in the reperfusion injury, however, the reports of the effect of cerebral ischemia on SOD expression and activity are contradictory [25,30]. A small decrease in SOD activity has been reported in most of the studies, including human acute cerebral ischemia [29]. Propofol has not demonstrated any effect on SOD activity [16,21], for that reason we did not measure this activity.

A further issue worthy of consideration is the range of concentrations in which propofol affects oxidative stress in brain tissue. After anaesthesia with habitual doses, plasma concentrations in humans [6] range from 40 to 80 μmol/l; the dose of 50 μmol/l tested in the present study, thus approximates that used in clinical practice, although caution is needed in comparing data from rats and humans. We found that at 50 μmol/l, propofol had significant effects on all parameters of oxidative stress studied here. We conclude that at concentrations used to induce anaesthesia in humans, propofol showed antioxidant effects in rat brain slices subjected to anoxia-reoxygenation in vitro. This effect may be of benefit in patients with antecedents of cerebral vascular ischemia who require general anaesthesia. However, further research in humans will be needed to confirm the ability of propofol to protect brain tissue from oxidative stress.

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References


