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Research Report

Neuroprotective effect of propofol on necrosis and apoptosis following oxygen–glucose deprivation—Relationship between mitochondrial membrane potential and mode of death

Takehiko Iijima[a,*], *Tatsuya Mishima*[b], *Kimio Akagawa*[b], *Yasuhide Iwao*[a]

[a] *Department of Anesthesiology, Kyorin University, School of Medicine, 6-20-2 Shinkawa Mitaka City, Tokyo 181-8611, Japan*
[b] *Department of Cell Physiology, Kyorin University, School of Medicine, 6-20-2 Shinkawa Mitaka City, Tokyo 181-8611, Japan*

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ABSTRACT

Mitochondrial membrane potential (MMP) appears to play an important role in apoptotic cascade and has been proposed as an index for apoptosis or necrosis. We examined the neuroprotective effect of propofol on mode of death, focusing on MMP. Hippocampal cell culture was divided into three groups: control, oxygen–glucose deprivation for 30 min (30OGD), 90 min (90OGD). Propofol was added to each culture group at a concentration of 0 μM (Vehicle), 0.1 μM (Pro0.1) or 1.0 μM (Pro1.0). MMP was expressed as normalized JC-1 fluorescence. ATP content was assayed using the luciferin–luciferase reaction. Neuronal viability and appearance of apoptosis were also assessed. ATP content was decreased after OGD (0.276 ± 0.115 μM/μg (control), 0.172 ± 0.125 μM/μg (30OGD) and 0.096 ± 0.092 μM/μg (90OGD)). Propofol did not alter ATP content. MMP was hyperpolarized after 30OGD (1.26 ± 0.23 (vehicle), 1.29 ± 0.13 (Pro0.1) and 1.18 ± 0.06 (Pro1.0)) but was depolarized after 90OGD (0.77 ± 0.04 (vehicle), 0.89 ± 0.04 (Pro0.1), but Pro1.0 prevented depolarization (1.03 ± 0.15 ($P < 0.05$)). Viability of cells significantly decreased to 50.3 ± 5.7% (vehicle), 46.1 ± 7.5% (Pro0.1), but Pro1.0 significantly salvaged neurons 65.1 ± 6.2% (higher than vehicle and Pro0.1 value, $P < 0.05$) after 90OGD. At 24 h after OGD, TUNEL-positive cells were increased to 34.5 ± 6.2% (vehicle), 26.7 ± 7.9% (Pro0.1) and 30.4 ± 7.1% (Pro1.0) in the 30OGD group. No pharmacological effect of propofol on the incidence of apoptosis was found. Propofol inhibited acute neuronal death accompanied with the maintenance of MMP but did not prevent subsequent apoptosis. Propofol induces a moratorium on neuronal death, during which pharmacological intervention might be able to prevent cell death.

1. Introduction

Many studies have reported that anesthetics have pharmacological protective effects against ischemic neuronal death. Neuronal protective effects might be exerted through the putative mechanism of NMDA receptor antagonism, and/or free radical scavenging, and/or reduction of energy consumption. Propofol has been found to exert neuroprotective effects through increased uptake of glutamate (Peters et al., 2001; Daskalopoulos et al., 2001; Velly et al., 2003) and scavenging of peroxynitrite (Acquaviva et al., 2004; Mathy-Hartert et al., 2000). The impact of ischemia and subsequent reperfusion is manifested as neuronal depolarization, and if it is prolonged, neurons become necrotic. Such acute necrosis is the final result

* *Corresponding author*. Fax: +81 422 43 1504.
E-mail address: iijmt@kyorin-u.ac.jp (T. Iijima).

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of ischemia and cannot be counteracted pharmacologically. Neuroprotection can be divided into two strategies: mitigation of the impact of ischemia, as with use of antioxidants to prevent acute necrosis, and prevention of subsequent expansion of neuronal death after ischemia as inhibition of apoptotic cascade. Recent study of apoptosis has revealed that mitochondria play an important role in the apoptotic cascade (Kroemer and Reed, 2000; Susin et al., 1997). Clarification of the effect of propofol on the mitochondrial pathway is important in examining the neuroprotective properties of this agent. Mitochondrial membrane potential (MMP) appears to be one of the regulators of the mitochondrial apoptotic cascade. Our laboratory has reported that MMP exhibits a specific alteration after oxygen–glucose deprivation (OGD) (Iijima et al., 2003a,b) linked to subsequent apoptosis (Iijima et al., 2003a,b). Although the pathophysiological effects of hyperpolarization of mitochondria (MHP) have not been determined, MHP has been observed in various pathological conditions (Kim et al., 2003; Perl et al., 2004; Perry et al., 2005). The mitochondrial hypothesis of neuronal death involves an earlier mechanism of ischemic neuronal death, which may open a therapeutic window for pharmacological intervention. We examined whether or not propofol prevents apoptosis after energy substrate depletion.

2. Results

2.1. ATP content

Control ATP contents were 0.268 ± 0.17 μM/μg protein (vehicle), 0.276 ± 0.115 μM/μg (Pro0.1) and 0.203 ± 0.133 μM/μg (Pro0.1). ATP concentration thus decreased depending on the duration of OGD. ATP contents were 0.172 ± 0.125 μM/μg (vehicle), 0.206 ± 0.132 μM/μg (Pro0.1) and 0.203 ± 0.120 μM/μg (Pro1.0) after 30 min OGD and further decreased to 0.096 ± 0.092 μM/μg (vehicle), 0.108 ± 0.115 μM/μg (Pro0.1) and 0.136 ± 0.121 μM/μg (Pro1.0) after 90 min OGD (Fig. 1).

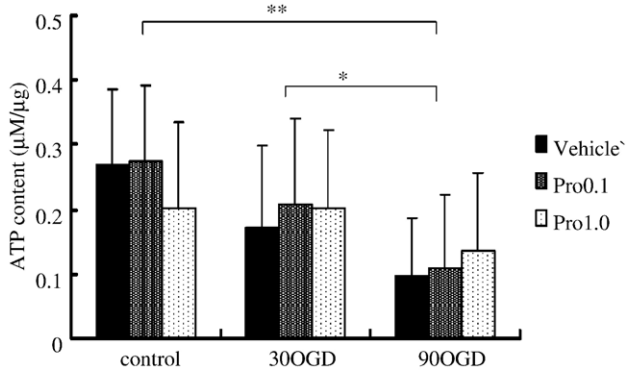

Fig. 1 – ATP content in each experimental condition. ATP content (mean ± SD) decreased depending on the length of OGD. Although addition of propofol to cell culture tended to preserve ATP, there was no significant difference in ATP content between either Pro0.1 and Pro1.0 and vehicle incubation. Abbreviations: 30OGD: 30 min oxygen–glucose deprivation (OGD), 90OGD: 90 min OGD, Pro0.1: propofol 0.1 μM, Pro1.0: propofol 1.0 μM. There was a significant difference between groups. *$P < 0.05$, **$P < 0.01$.

2.2. Mitochondrial membrane potential

Propofol did not influence MMP in control condition (1.0 (control), 0.98 ± 0.05 (Pro0.1) and 1.06 ± 0.04 (Pro1.0), $P > 0.05$). MMP was hyperpolarized after 30 min OGD (1.26 ± 0.23 (vehicle), 1.29 ± 0.13 (Pro0.1) and 1.18 ± 0.06 (Pro1.0)) (normalized fluorescence: 1.0 for control state). These values were significantly higher than the control value ($P < 0.001$). MMP decreased toward depolarization after 90 min OGD (0.77 ± 0.04 (vehicle), 0.89 ± 0.04 (Pro0.1) and 1.03 ± 0.15 (Pro1.0)). These values were significantly lower than the control value ($P < 0.001$) (Fig. 2). The intra-group differences between vehicle, Pro0.1 and Pro1.0 within 90 min OGD group were observed (vehicle vs. Pro0.1; $P = 0.015$, vehicle vs. Pro1.0; $P < 0.001$, Pro0.1 vs. Pro1.0; $P = 0.041$). The MMP value of depolarization was formerly obtained in the previous article (Iijima et al., 2003a,b) (0.66 ± 0.22, valinomycin (potassium ionophore)).

2.3. Viability

Viabilities of cells in the control were 85.5 ± 3.7% (vehicle), 85.9 ± 1.9% (Pro0.1) and 81.9 ± 1.5% (Pro1.0). After 30 min OGD, viability significantly decreased to 70.9 ± 13.0% (vehicle), 66.1 ± 10.6% (Pro0.1) and 67.4 ± 9.8% (Pro1.0). After 90 min OGD, viability further decreased to 50.3 ± 5.7% (vehicle), 46.1 ± 7.5% (Pro0.1) and 65.1 ± 6.2% (Pro1.0). We found a statistically significant decrease in viable cell ratio with duration of OGD (ANOVA, $P < 0.001$) and with pharmacological intervention (Fig. 3). As intra-group difference, significant difference (MANOVA, $P < 0.05$) was observed between vehicle and Pro1.0 in 90OGD group.

2.4. Apoptosis

At 3 h after OGD, TUNEL-positive cells were detected from 6.8% to 13.3% after 30 min OGD, from 16% to 17% after 90 min OGD. Even the control featured TUNEL-positive cells, at 2% to 4%. These values were significantly different between durations ($P < 0.001$) (Fig. 4).

At 24 h after OGD, TUNEL-positive cells increased to 34.5 ± 6.2% (vehicle), 26.7 ± 7.9% (Pro0.1) and 30.4 ± 7.1% (Pro1.0) after 30 min OGD. TUNEL-positive cells in the 90 min OGD group were 11.4 ± 4.1% (vehicle), 18.0 ± 10.3% (Pro0.1) and 9.3 ± 3.1% (Pro1.0). In the control cultures, TUNEL-positive cells were detected at 2.4 ± 2.8% (vehicle), 6.9 ± 5.5% (Pro0.1) and 16.3 ± 14.7% (Pro1.0) (Fig. 5).

3. Discussion

We used 30OGD and 90OGD models to mimic graded ischemic insult; 30OGD corresponds to mild ischemia, inducing apoptosis, while 90OGD corresponds to strong ischemia, inducing necrosis. Our previous investigation showed that the former insult induces hyperpolarization of MMP, and the latter depolarization of MMP (Iijima et al., 2003a,b). We employed this model to identify the process by which propofol intervenes in the death program.