Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology 225 (2006) 97–108
TOXICOLOGY
www.elsevier.com/locate/toxicol

Reciprocal effects of glucose on the process of cell death induced by calcium ionophore or H_2O_2 in rat lymphocytes

Kanna Horimoto, Yumiko Nishimura, Tomohiro M. Oyama, Kyoko Onoda, Hiroko Matsui, Toshihisa B. Oyama, Kaori Kanemaru, Toshiya Masuda, Yasuo Oyama *

Laboratories of Cell Signaling and Bioorganochemistry, Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770-8502, Japan

Received 18 March 2006; received in revised form 7 May 2006; accepted 12 May 2006
Available online 16 May 2006

Abstract

We have examined the effects of glucose at high concentrations on the process of cell death induced by excessive increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) or oxidative stress in rat lymphocytes. The cell death elicited by the excessive increase in $[Ca^{2+}]_i$ seemed to be induced by an activation of Ca^{2+}-dependent K^+ channels because the inhibitors for Ca^{2+}-dependent K^+ channels attenuated the decrease in cell viability. Glucose at 30–50 mM augmented the decrease in cell viability by the excessive increase in $[Ca^{2+}]_i$. It was not specific for glucose because it was the case for sucrose or NaCl, suggesting an involvement of increased osmolarity in adverse action of glucose. On the contrary, glucose protected the cells suffering from oxidative stress induced by H_2O_2, one of reactive oxygen species. It was also the case for fructose or sucrose, but not for NaCl. The process of cell death induced by H_2O_2 started, being independent from the presence of glucose. Glucose delayed the process of cell death induced by H_2O_2. Sucrose and fructose also protected the cells against oxidative stress. The reactivity of sucrose to reactive oxygen species is lower than those of glucose and fructose. The order in the reactivity cannot explain the protective action of glucose. Glucose at high concentrations exerts reciprocal actions on the process of cell death induced by the oxidative stress and excessive increase in $[Ca^{2+}]_i$.

Keywords: Cell death; Lymphocyte; Calcium; Oxidative stress; Glucose

1. Introduction

Reactive oxygen species (ROS) and Ca^{2+} are used as messengers and mediators in normal cell functions (Rasmussen et al., 1990; Khan and Wilson, 1995; Johnson et al., 1996; Berridge et al., 1998; Sauer et al., 2001). However, excessive oxidative stress by ROS and sustained increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) are insults that cause cell injury and/or death (Trump and Berezesky, 1995; Ichas and Mazat, 1998; Duchen, 1999; Anderson et al., 1999; Fleury et al., 2002). As to an oxidative stress, ROS play a dual role in the process of cell death: first, as a signal during induction phase, and second, as a common consequence of mitochondrial permeability transition leading to final cell destruction (Jabs, 1999). With respect to a sustained increase in $[Ca^{2+}]_i$, it exerts adverse effects by activating cellular proteases and lipases, leading to trigger either apoptotic or necrotic cell death (Kristian and Siesjo, 1998; Orrenius et al., 2003). Furthermore,

* Corresponding author. Tel.: +81 88 656 7256; fax: +81 88 656 7256.
E-mail address: oyama@ias.tokushima-u.ac.jp (Y. Oyama).

0300-483X/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2006.05.004

these two insults are linked to each other (Mattson et al., 1995; Oyama et al., 1996; Ermak and Davies, 2002). The oxidative stress induces an increase in $[Ca^{2+}]_i$ while the increase in $[Ca^{2+}]_i$ increases an oxidative stress in neurons.

Glucose is one of the most important carbohydrates. It is a ubiquitous fuel, oxidized to eventually form CO_2 and water through glycolysis and tricarboxylic acid cycle (TCA cycle). The TCA cycle takes the left-over carbon from glycolysis and breaks it down in such a way as to yield a large amount of useable energy, adenosine triphosphate. However, glucose induces oxidative stress, leading to cell injury and/or death in several types of cells or tissues (Sharpe et al., 1998; Greene et al., 1999; Gow et al., 1999; Catherwood et al., 2002; Folmer et al., 2002; Russell et al., 2002; Allen et al., 2003; Wu et al., 2004). Of ROS, hydrogen peroxide (H_2O_2) is involved in the oxidative stress induced by glucose (Ruiz-Mounoz et al., 1997; Panayiotidis et al., 1999; Peiro et al., 2001). It induces necrosis and/or apoptosis in several types of cells (Whittemore et al., 1994; de Bono and Yang, 1995; Bhat and Zhang, 1999; Kanno et al., 1999; Oyama et al., 1999). However, the process of cell death induced by H_2O_2 seems to vary from cells to cells (Vollgraf et al., 1999; Jiang et al., 2001; Dodo et al., 2005; Lee et al., 2005; Yu et al., 2005). The application of H_2O_2 induces an excessive increase on $[Ca^{2+}]_i$ in several types of cells, leading to cell death (Josephson et al., 1991; Ueda and Shah, 1992; Herson et al., 1999). Therefore, it is important to examine the effect of glucose on the cells suffering from oxidative stress or intracellular Ca^{2+} overload since high blood concentration of glucose is commonly observed in diabetic patients and the number of people with diabetes worldwide is expected to rise to well over 200 million by 2010 (Zimmet et al., 2001).

2. Materials and methods

2.1. Reagents

A23187 calcium salt, clotrimazole, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, USA). Both agents were initially dissolved in DMSO and then added to Tyrode's solution. Final concentration of DMSO as a solvent in Tyrode's solution was 0.1% or less. DMSO at 0.3% or less did not affect the viability of rat lymphocytes since the incubation of cells with this vehicle (0.01–0.3%) for 3 h did not increase the population of dead cells under control condition. Charybdotoxin was obtained from Peptide Institute (Osaka, Japan). Glucose and other chemicals except for fluorescent probes were purchased from Woko Pure Chemical (Osaka, Japan). The concentrations of glucose used in this study were 5–50 mM. Highest concentration (50 mM) of glucose is still lower than those observed in some diabetic patients (McDonnell et al., 2005). Furthermore, in previous studies (Li et al., 2001; Peiro et al., 2001; Russell et al., 2002; Wu et al., 2004), glucose at 20–100 mM was reported to induce cell death in smooth muscle cells, neurons, and pancreatic cells.

2.2. Animals

Male rats (Wistar strain) were provided with water automatically and a commercial diet (MF, Oriental Yeast, Tokyo, Japan) *ad libitum*. The animal room was maintained at a temperature of $23 \pm 2\,^{\circ}C$ and a relative humidity of $55 \pm 5\%$, and it was artificially illuminated with fluorescent light on a 12-h light/dark cycle (08:00–20:00 h). The total number of 3–4-week-old rat sacrificed under ether anesthesia was 32. This study was approved by the Committee for Animal Experiments in the University of Tokushima (Registered No. 05279).

2.3. Cell preparation

The procedure to prepare cell suspension was similar to that previously reported (Chikahisa and Oyama, 1992; Oyama et al., 1992). In brief, thymus glands dissected from 3- to 4-week-old Wistar rats were sliced at a thickness of 400–500 μm. The slices were triturated in chilled Tyrode's solution (NaCl 150 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, glucose 5 mM, HEPES 5 mM, with an appropriate amount of NaOH to adjust pH to 7.3–7.4) to dissociate lymphocytes. Thereafter, the Tyrode's solution was passed through a mesh (a diameter of 10 μm) to prepare the cell suspension (about 5×10^5 cells/ml). The cells were incubated at 35–36 °C for 1 h before use.

2.4. Experimental design

The test agents were added to the cell suspension (2 ml). The cells were incubated with the agent(s) at 35–36 °C for 1–3 h. The data acquisition of fluorescence from 2500 cells by a flow cytometer required 30 s. The fluorescence was analyzed by JASCO software (Ver. 3XX, JASCO, Tokyo, Japan).

2.5. Fluorescence measurements of cellular parameters

The methods for measurements of cellular parameters using a flow cytometer equipped with an argon laser (CytoACE-150, JASCO, Tokyo, Japan) and fluorescent probes to monitor cellular parameters were similar to those described previously (Chikahisa and Oyama, 1992; Oyama et al., 1992; Chikahisa et al., 1996).

To assess the cell viability, propidium iodide (Molecular Probe Inc., Eugene, OR, USA) was added to cell suspension to achieve a final concentration of 10 μM. Since propidium stains dead cells, the measurement of propidium fluorescence from the cells provides a clue to estimate the viability. Propidium fluorescence was measured at 1–2 min after the application by a flow cytometer. Excitation wavelength for