



0006-2952(94)00401-3

EFFECT OF THIMEROSAL AND OTHER SULFHYDRYL REAGENTS ON CALCIUM PERMEABILITY IN THYMUS LYMPHOCYTES

ELIZABETH PINTADO,* DOMINGO BAQUERO-LEONIS, MANUEL CONDE and FRANCISCO SOBRINO

Departamento de Bioquímica Médica y Biología Molecular, Laboratorio Sistemas Inmunológicos, Facultad de Medicina, Universidad de Sevilla, Avda Sanchez-Pizjuan 4, 41009 Sevilla, Spain

(Received 23 March 1994; accepted 29 August 1994)

Abstract—We have studied the effects of thimerosal, a mercurial compound extensively used as a preservative, as well as other sulfhydryl reagents (e.g. *p*-hydroxymercuribenzoate, hydrogen peroxide, bromophenacyl bromide, and mercuric chloride) on Ca^{2+} homeostasis and the redox status of sulfhydryl groups in thymus lymphocytes. They all induced an increase in $[\text{Ca}^{2+}]_i$, which was blocked with dithiothreitol, suggesting that they act via the oxidation or blockade of sulfhydryl groups. $[\text{Ca}^{2+}]_i$ increase could be directly related to the effect of the different reagents on cellular protein sulfhydryl content. Experiments with ethidium bromide indicate that the observed rise in $[\text{Ca}^{2+}]_i$ was not due to a non-specific increase in membrane permeability. Thimerosal differs from the other agents studied in its oxidative properties, which is probably linked to the production of a potent reductor molecule, thiosalicic acid, which may modulate its oxidative capacity.

Key words: Ca^{2+} homeostasis; thymus lymphocytes; thimerosal; sulfhydryl reagents

Thimerosal is used as a preservative in many pharmaceutical solutions and has also been reported to elicit the production of specific antibodies and cell-mediated immunity [1, 2]. These effects are related to its oxidative capacity [2]. The redox status of a cell is an important factor in the maintenance of general cellular homeostasis, in particular calcium homeostasis [3–7]. Essential sulfhydryl groups are present in a number of membrane-bound proteins related to Ca^{2+} permeability, and it is known that sulfhydryl reagents may alter the internal Ca^{2+} concentration [8–10]. We have previously described that thimerosal induces an increase in $[\text{Ca}^{2+}]_i$ in rat thymus lymphocytes [11], as well as in other cell preparations [12–19].

The effect of thimerosal on $[\text{Ca}^{2+}]_i$ homeostasis is complex, varying with dose and cell type [15–20]. In some cases, it has been proposed that thimerosal induces an increase in $[\text{Ca}^{2+}]_i$ by sensitizing the InsP_3 receptor, a phenomenon also observed with oxidized glutathione and *t*-butyl hydroperoxide at higher concentrations [18, 21]. In other preparations, thimerosal seems to open a pathway for Ca^{2+} entry from the extracellular side [11, 19].

The present study analyses the effects of thimerosal and other sulfhydryl reagents, including BPB, H_2O_2 , HgCl_2 and pHMB,† on $[\text{Ca}^{2+}]_i$ in thymus lymphocytes. Although the compounds used differed

in their mechanisms of action, their effects in all cases were abolished with DTT. We provide evidence that there is a relationship between the SH-blocking capacity and the potency of these agents in increasing $[\text{Ca}^{2+}]_i$. These effects are not a general consequence of cell damage since cell viability as evidenced by LDH release and ethidium bromide uptake was not affected by brief exposure to thiol reagents.

MATERIALS AND METHODS

Cell isolation. Thymocytes were prepared from 6-week-old Wistar rats of either sex as previously described [11], and kept in a standard saline composed of (in mmol/L): 125 NaCl, 5 KCl, 1 CaCl₂, 2 MgCl₂, 1.5 NaH₂PO₄, 10 glucose and 25 Hepes, pH 7.4. Standard saline was used in all experiments unless otherwise indicated.

Measurement of $[\text{Ca}^{2+}]_i$ concentration. Loading cells with Fura-2/AM and measurement of $[\text{Ca}^{2+}]_i$ were performed as previously described [11]. For measurement of $[\text{Ca}^{2+}]_i$, thymocytes were suspended in a thermostatically controlled and magnetically stirred fluorimeter cuvette (Perkin-Elmer LS5) at a concentration of $1-2 \times 10^7$ cells/mL. Since phosphate, when complexed with manganese, increases autofluorescence at the excitation wavelength of Fura-2, free phosphate was omitted from the reaction mixture in all experiments. To prevent Fura-2 leakage from the cells we used 2.5 mM probenecid. Fura-2 leakage was determined in the calibration cuvettes by the decrease in fluorescence after the addition of 100 μM MnCl₂. Only cell preparations with less than 1–2% fluorescence decrease (100 is the arbitrary unit for fluorescence in the presence of digitonin) were used for the experiments.

* Corresponding author. FAX 34-5-4907041.

† Abbreviations: AA, arachidonic acid; BPB, bromophenacyl bromide; $[\text{Ca}^{2+}]_i$, internal calcium concentration; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); DTT, dithiothreitol; *p*-HMB, *p*-hydroxymercuribenzoate; LDH, lactate dehydrogenase; 2-ME, 2-mercaptoethanol; PHA, phytohaemagglutinin; PLA₂, phospholipase A₂; SH, sulfhydryl; TNB, 2-nitro-5-thiobenzoic acid.