



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

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(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  $\text{Ca}^{2+}$  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:**  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  permeability, and it is known that sulfhydryl reagents may alter the internal  $\text{Ca}^{2+}$  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  $\text{InsP}_3$  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  $\text{Ca}^{2+}$  entry from the extracellular side [11, 19].

The present study analyses the effects of thimerosal and other sulfhydryl reagents, including BPB,  $\text{H}_2\text{O}_2$ ,  $\text{HgCl}_2$  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  $\text{CaCl}_2$ , 2  $\text{MgCl}_2$ , 1.5  $\text{NaH}_2\text{PO}_4$ , 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  $1\text{--}2 \times 10^7$  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  $\mu\text{M}$   $\text{MnCl}_2$ . 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.

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† 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;  $\text{PLA}_2$ , phospholipase  $\text{A}_2$ ; SH, sulfhydryl; TNB, 2-nitro-5-thiobenzoic acid.