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Na^+/Ca^{2+} Exchange-mediated La^{3+} Entry in Human Lymphocytes

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Abstract: We determined whether La^{3+} enter human peripheral blood lymphocytes *via* Na^+/Ca^{2+} exchanger (measured with fura-2). We first compared the sensitivity of fura-2 with La^{3+} and Ca^{2+}, the result indicates that the sensitivity of fura-2 for La^{3+} is much greater than for Ca^{2+}. La^{3+} forms a 1:1 La^{3+}-fura-2 complex (apparent dissociation constant $=1.7\times10^{-12}$mol/L, pH 7.05). Ouabain-pretreated cells, suspended in Na^+-free medium, showed that La^{3+} can enter human lymphocytes *via* the Na^+_i/Ca^{2+} $(La^{3+})_o$ exchanger and is found to be about 10^{-12} mol/L in cells exposed to 0.4 mmol/L La^{3+}. Otherwise, the higher concentration (0.1 mmol/L) blocks the $Na^+_i/Ca^{2+}(La^{3+})_o$ exchange–mediated influx of Ca^{2+}, but the lower concentration (0.01 mmol/L) appears to increase Ca^{2+} entry.

Keywords: Na^+/Ca^{2+} exchanger, lymphocytes, La^{3+}, ouabain, fluorescence indicator.

With extensive applications the rare earth elements to agriculture, medicine and animal husbandry, whether rare earth ions can cross cell membranes and participate in the metabolism of animals and plants is not clear until now. Otherwise, rare earth ions have different interaction fashions to different kinds of cells, therefore, in this study we determined whether La^{3+} enter human peripheral blood lymphocytes *via* Na^+/Ca^{2+} exchanger (measured with fura-2).

Relative sensitivity of fura-2 to Ca^{2+} and La^{3+}

We first compared the sensitivity of 0.06 μ mol/L fura-2 with La^{3+} and Ca^{2+} in solutions simulating intracellular ionic composition. The results strongly suggest that the sensitivity of fura-2 for La^{3+} and Ca^{2+} is 10^{-12} mol/L and 10^{-8} mol/L, respectively. Fluorescence spectra of La^{3+} and fura-2 mixtures were analyzed by a Hill plot[1], *i.e.*, plotting log (fura-2 bound/ fura-2 free) *vs*. log[La^{3+}], such a plot yields a straight line, indicating a 1:1 stoichiometry of the La^{3+}-fura-2 complex, the apparent dissociation constant (K_d) for the La^{3+}-fura-2 complex (calculated from the y-intercept value) obtained in this particular experiment was 1.7×10^{-12} mol/L at pH 7.05.

La^{3+} uptake by Na^+/Ca^{2+} exchange-mediated in lymphocytes

The Na^+/Ca^{2+} exchanger is a carrier system that mediated the transport of Ca^{2+} across the membrane in direct exchange for Na^{+2}. In most cells expressing Na^+/Ca^{2+} exchange activity, the physiological role of this system is to pump Ca^{2+} out of the cell ("forword mode" Na^+_o/Ca^{2+}_i exchange); however, under conditions where cytosolic Na^+ is increased, the exchanger can carry out the net influx of Ca^{2+} ("reverse mode" Na^+_i/Ca^{2+}_o exchange).

To determine whether La^{3+} enter lymphocytes by Na^+/Ca^{2+} exchanger, lymphocytes was exposed to ouabain and 3 μ mol/L fura-2-AM in Na^+ medium for 30 min at 37°C. Then cells were centrifuged to remove external fura-2 and resuspended in Na^+-free

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(NaCl was replaced by LiCl), Ca^{2+}-free containing 0.1mmol/L EGTA medium, the 340/380 nm fluorescence ratio were continuously measured. After La^{3+} was added, the results indicate that the rise of the 340/380nm fluorescence ratio is dependent on $[La^{3+}]_o$, but it is also shown that $[La^{3+}]_o$, at concentrations >0.4 mmol/L, can not cause the change of the fluorescence ratio again. The data imply that La^{3+} at a certain threshold concentration limits its own entry *via* the $Na^+_i/Ca^{2+}(La^{3+})_o$ exchanger[3]. 1.0 mmol/L ouabain-treated cells showed an increase in fluorescence ratio compared to 0.5 mmol/L ouabain-treated cells, it is suggested that both an increase in cytosolic $[Na^+]$ and reversal of the Na^+ gradient are necessary to demonstrate La^{3+} influx in lymphocytes. According to the literature 1, a formula can be derived for calculation of La^{3+} concentration as a function of ratios of fluorescence:

$$[La^{3+}]=K_d\ (La)\ [(R_a-R_b)(R_{maxCa}-R_{min})Sf_2]/[(R_{maxLa}-R_a)(R_{maxCa}-R_b)\ Sb_{2La}]$$

In each experiment, the maximum and minimum fluorescence ratios were sequentially determined according to Chiu-Yin Kwan *et al*.[4]. R_a and R_b were the ratio of the 340/380 nm fluorescence after and before the addition of La^{3+} respectively. Cells autofluorescence was determined with suspensions of cells not loaded with fura-2 and was subtracted from the traces before $[La^{3+}]$ was calculated.

Thus, fluorescence data were transformed into La^{3+} concentrations using this equation. La^{3+} is found to be about 10^{-12} mol/L in cells exposed to 0.4 mmol/L La^{3+}.

Effects of La^{3+} on Ca^{2+} entry by Na^+/Ca^{2+} exchanger

La^{3+} exhibits several properties similar to that of calcium in biological systems presumably by virtue of its comparable ionic radius. For example, La^{3+} displaces calcium from its binding site on the external surface of the plasma membrane and blocks calcium influx and efflux from cells[5,6]. Ouabain-pretreated cells, suspended in Na^+-free 1mmol/L Ca^{2+} medium, showed an increase in Ca^{2+} uptake in a rapid and saturating fashion. This uptake of Ca^{2+} was inhibited by the presence of 0.1mmol/L La^{3+}. At this concentration, La^{3+} enters cells, so La^{3+} displaces calcium from Na^+/Ca^{2+} exchanger site and blocks calcium influx[7]. But low concentration of La^{3+} (0.01mmol/L) increased calcium influx. As La^{3+}, at this concentration, is not found to cause change of the 340/380nm fluorescence ratio, La^{3+} may bind to the plasma membrane Ca-ATPase to account for its inhibition of Ca efflux which showed an increase of Ca^{2+} entry[8].

In conclusion, it is clear that La^{3+} can be transported by Na^+/Ca^{2+} exchanger, but at higher concentration (0.1mmol/L), La^{3+} blocks the exchanger, low concentration of La^{3+} increase Ca^{2+} influx by Na^+/Ca^{2+} exchanger.

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