J. Biol. Res. 2002 n. 1 - Vol. LXXVIII - Rubbettino - Soveria Mannelli

Action of Ion Flux Regulators on Membrane of Liver Cells in Primary Culture

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Introduction

The present paper deals with the interaction of some ion flux regulators and metabolic inhibitors upon the generation of the membrane potential in isolated liver cell culture. This study is the basis of an accurate selection of inhibitors of specific cellular metabolic pathways, which control the ion transport of membrane. This selection will allow to improve both the fluorescence microscopy study methods of membrane transport and the evaluation techniques concerning the action of some metabolic modulators such as the hormones.
In this paper, the following compounds are tested: Alameticin, a channel forming ionophor composed by a linear sequence of 20 aminoacids (1); Lasalocid, a carboxylic ionophor (2); and FCCP, uncoupler of oxydative phosphorilation.

Materials and methods

In our experiments, male rats from the Wistar strain, 90 days old, have been used after ether anasthesia, the abdomen has been cut and, after locating the portal vein or the inferior vena cava, the liver perfusion has been carried out and the organ has been explanted and lysed according to Seglen method (3) (4).
On the resulting lysate, the cell count has been performed through optical microscopy. The cell cultures have been prepared using Eagle medium, modified by Dulbecco, with 10% bovine foetal serum addition, in multiwell chambers .
In these cultures the compounds to be tested, as well as the fluorescent probe Propidium Iodide (from Molecular Probes, USA) have been added in order to allow the observation of the cells by fluorescence microscopy. This probe has also great affinity with the DNA double helix, but at concentrations higher than 20 μM it's also present in the cytoplasm where it establishes electrostatic interaction with the intracellular environment (5).
The cell cultures have been observed by fluorescence microscopy and then transferred on a PC by a high definition camera, using the software Image Pro Plus 4.0. Then the images have been analysed using the software NIH Image 1.60 program. This analysis has provided the cytoplasm areas and the cumulative fluorescence integral has been referred to the value of the corresponding area. The resulting values have been expressed as relative per cent fluorescence, at zero and three hours of incubation with the fluorescent label, and represented in hystogram as function of either the probe, and the ionophores and the uncoupler concentrations.
To analyse the effects produced by the tested compounds, the optimal concentration of propidium iodide (16 μM) has been established in order to attain the ideal conditions for our research. Then the compounds have been tested at the following final concentrations: 1-12,5-15-12-21-24 μM.

Results

Figure 1 shows the trend of the relative per cent fluorescence of the probe (16 μM) with Alameticin at 12,5 μM concentration in comparison with reference experiments, and has proved to be the best one in order to generate the membrane potential depolarization.

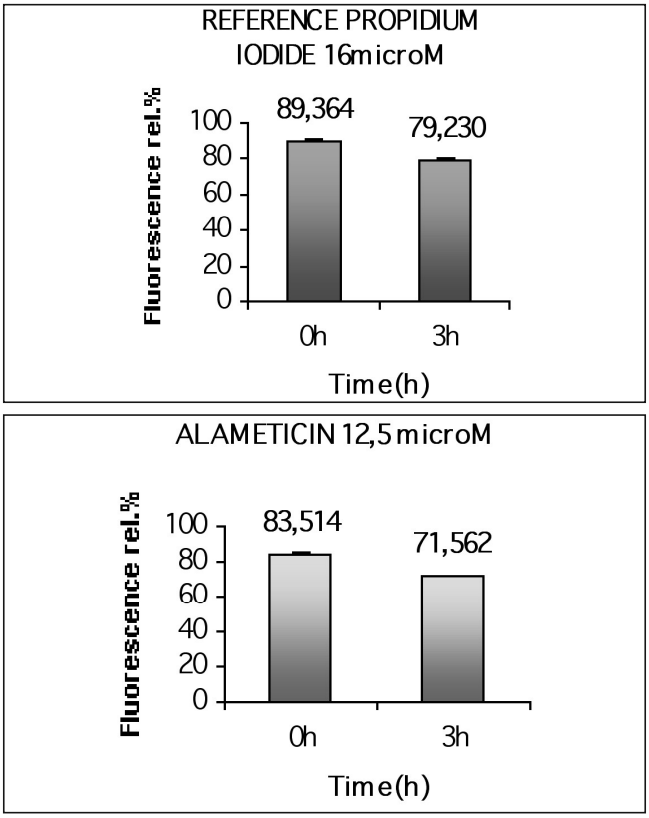

Fig. 1 - Trend of the Alameticin relative per cent fluorescence, compared to the reference test carried out in parallel. Each result is the mean of 7 independent determinations . T: S.E.M.

Fig. 2 shows the action produced by Lasalocid upon the generation of the membrane potential at 15 μM concentration, which decreases the relative per cent fluorescence of about 20% within three hours in comparison to reference data .

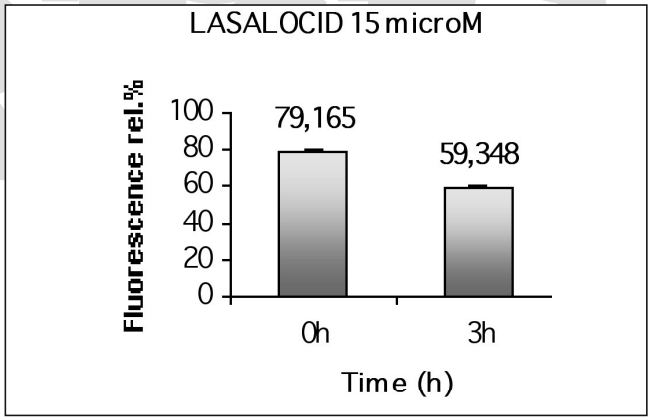

Fig. 2. Trend of the Lasalocid relative per cent fluorescence, compared to the reference test carried out in parallel. Each result is the mean of 7 independent determinations . T: S.E.M.

Finally, Fig. 3 shows the trend of the relative per cent fluorescence with FCCP at 1 μM concentration, which has proved to be the most effective one, since it has produced a variation of about 20%, in comparison to reference tests.

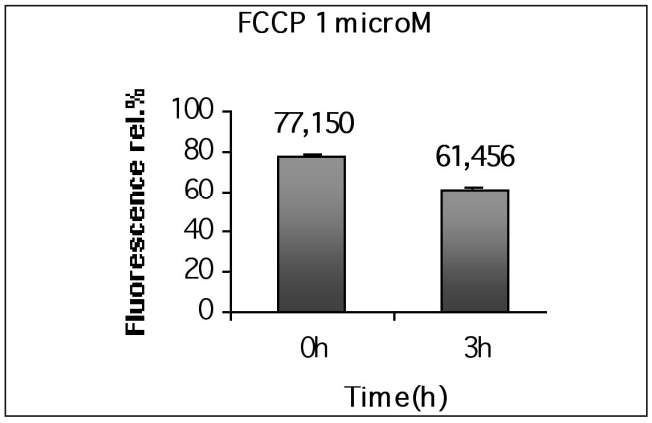

Fig. 3. Trend of the FCCP relative per cent fluorescence, compared to the reference test carried out in parallel. Each result is the mean of 7 independent determinations . T: S.E.M.

Discussion

The results of our research are useful to study non excitable cells, through the image analysis technique.
The contemporary use of the analysis method with some of the ionophores or with oxydative phosphorilation uncoupling agents allows to appreciate the contribution that every single metabolic process gives to the ionic transport of plasma membrane.
Among the tested compounds, the highest membrane depolarization is produced by the carrier Lasalocid.
The depolarizing effect can be mainly attributed to the selectivity towards the K+ ion, according to the selectivity of the used carriers, or even to the effect of FCCP upon the transport of H+ which, inside the cell, can discharge the mitochondrial proton gradient.

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