Measurement of Free Cytosolic Calcium Concentration ([Ca^{2+}]i) in Single CHO-K1 Cells

Manuel D. Gahete, Raúl M. Luque* and Justo P. Castaño*

Department of Cell Biology, Physiology and Immunology, University of Córdoba, Reina Sofía University Hospital, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), and CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Córdoba, Spain
*For correspondence: raul.luque@uco.es; justo@uco.es

[Abstract] This is a protocol to analyze the functional response of single CHO-K1 cells to a given treatment in terms of changes in free cytosolic calcium concentration ([Ca^{2+}]i). This is possible by using the Ca^{2+} indicator dye Fura-2 AM, a polyamino carboxylic acid that binds to free intracellular calcium and is excited at 340 nm and 380 nm. The ratio of the emissions at 505 nm after excitation with those wavelengths is directly correlated to the amount of intracellular calcium. This protocol can be applied to other cell types (cell lines or primary cell cultures) by changing the culture conditions accordingly to the cell type.

Materials and Reagents

1. Ca^{2+} indicator dye Fura-2-acetoxymethyl ester (Fura-2 AM) (Molecular Probes)
2. Phenol red-free DMEM
3. $NaHCO_3$

Equipment

1. 25 mm round glass coverslips
2. Nikon Eclipse TE200-E microscope with attached back thinned-CCD cooled digital camera (ORCAII BT; Hamamatsu Photonics)

Software

1. MetaFluor Software (Imaging Corp)

Procedure

1. Plate 50000 CHO-K1 cells/ml onto 25 mm round glass coverslips for 24 h.

2. Load the cells with 2.5 µM of the Ca^{2+} indicator dye Fura-2 AM (Molecular Probes) in phenol red-free DMEM containing 20 mM $NaHCO_3$ (pH 7.4).
3. Incubate 30 min at 37 °C.
4. Wash with phenol red-free DMEM and incubate in phenol red-free DMEM containing 20 mM $NaHCO_3$ (pH 7.4) for 15 min (to allow the hydrolysis of the ester group).
5. Mount the round glass coverslip with the cells on the stage of a Nikon Eclipse TE200-E microscope.
6. Add 300 µl of phenol red-free DMEM containing 20 mM $NaHCO_3$ (pH 7.4).
7. Localize the cells to be recorder (as many as possible) and mark them as well as 2-3 background regions using the MetaFluor Software.
8. Acquire images of the loaded cells under a x40 oil immersion objective during exposure to alternating 340- and 380-nm light beams, and measuring the intensity of light emission at 505 nm every 5 sec.
9. After 30-40 sec where the baseline is stablished, add the treatment (300 µl) whose effect want to be proven, and continue recording.
10. Changes in $[Ca^{2+}]i$ after treatment administration are recorded as background substrates ratios of the corresponding excitation wavelength (F340/F380) using MetaFluor Software.

Acknowledgments

This protocol is adapted from Cordoba-Chacon *et al*. (2010); Cordoba-Chacon *et al*. (2011) and Duran-Prado *et al*. (2012).

References

1. Cordoba-Chacon, J., Gahete, M. D., Duran-Prado, M., Pozo-Salas, A. I., Malagon, M. M., Gracia-Navarro, F., Kineman, R. D., Luque, R. M. and Castano, J. P. (2010). Identification and characterization of new functional truncated variants of somatostatin receptor subtype 5 in rodents. *Cell Mol Life Sci* 67(7): 1147-1163.
2. Cordoba-Chacon, J., Gahete, M. D., Pozo-Salas, A. I., Martinez-Fuentes, A. J., de Lecea, L., Gracia-Navarro, F., Kineman, R. D., Castano, J. P. and Luque, R. M. (2011). Cortistatin is not a somatostatin analogue but stimulates prolactin release and inhibits GH and ACTH in a gender-dependent fashion: potential role of ghrelin. *Endocrinology* 152(12): 4800-4812.
3. Duran-Prado, M., Bucharles, C., Gonzalez, B. J., Vazquez-Martinez, R., Martinez-Fuentes, A. J., Garcia-Navarro, S., Rhodes, S. J., Vaudry, H., Malagon, M. M. and Castano, J. P.

(2007). Porcine somatostatin receptor 2 displays typical pharmacological sst2 features but unique dynamics of homodimerization and internalization. *Endocrinology* 148(1): 411-421.

4. Duran-Prado, M., Gahete, M. D., Hergueta-Redondo, M., Martinez-Fuentes, A. J., Cordoba-Chacon, J., Palacios, J., Gracia-Navarro, F., Moreno-Bueno, G., Malagon, M. M., Luque, R. M. and Castano, J. P. (2012). The new truncated somatostatin receptor variant sst5TMD4 is associated to poor prognosis in breast cancer and increases malignancy in MCF-7 cells. *Oncogene* 31(16): 2049-2061.
5. Martinez-Fuentes, A. J., Moreno-Fernandez, J., Vazquez-Martinez, R., Duran-Prado, M., de la Riva, A., Tena-Sempere, M., Dieguez, C., Jimenez-Reina, L., Webb, S. M., Pumar, A., Leal-Cerro, A., Benito-Lopez, P., Malagon, M. M. and Castano, J. P. (2006). Ghrelin is produced by and directly activates corticotrope cells from adrenocorticotropin-secreting adenomas. *J Clin Endocrinol Metab* 91(6): 2225-2231.