REVISTA ESPAÑOLA DE FISIOLOGIA, 46 (3), 237-240, 1990

Glucose Release in Mantle Tissue of Mytilus: Regulation by Calcium Ions

C. A. Crespo* and J. Espinosa

Departamento de Fisiología
Facultad de Farmacia
Universidad de Santiago de Compostela
15706 Santiago de Compostela (Spain)

(Received on May 19, 1989)

C. A. CRESPO and J. ESPINOSA. *Glucose Release in Mantle Tissue of Mytilus: Regulation by Calcium Ions.* Rev. esp. Fisiol., 46 (3), 237-240, 1990.

Glucose release activity in mantle tissue of *Mytilus galloprovincialis* was studied. Mantle tissue shows a basal glucose releasing activity. The external Ca^{2+} absence increases 2 to 3-fold the basal glucose release, and when A23187 (10 μM) was simultaneously present the release doubled that obtained in Ca^{2+}-absence. EGTA (2 mM), chlorpromazine (200 μM) and lanthanum (3 mM) decreased the glucose release promoted by external Ca^{2+} absence. This and other data suggest that glucose release activity in mantle tissue might be controlled by Ca^{2+} ions.

Key words: *Mytilus galloprovincialis*, Glucose release, Calcium ions.

In Mytilus mantle tissue is composed of two types of cells, vesicular (VC) and adipogranular (ADG). In this tissue glycogen is the most important and constitutive bioenergetic reserve material, which is mainly stored in VC and to a lesser extent in ADG (3, 9, 10, 13). Moreover, mantle tissue is invaded by the gonad during the reproductive cycle, where the germinal cells are distributed in an irregular manner among VC and ADG (14, 15), and where the glycogen stores show a clear periodicity in their content (6, 7, 9). This periodicity in the mantle glycogen content and the heterogeneity of this tissue suggest that the polyssacharide reserves might be distributed asymmetrically all over the mantle.

Little is known about the mechanisms that control the glycogen breakdown in Mytilus, and about the existence of a glucose transference from glycogen storage areas to the other ones where this monosaccharide is needed for use in other metabolic pathways.

In the present paper we suggest a glucose releasing activity in mantle fragments when the glycogenolytic cascade is affected by Ca^{2+} ions as has already been demonstrated in mammals (2, 8, 17).

* To whom all correspondence should be addressed.

Materials and Methods

Animals.— Sea mussels, *Mytilus galloprovincialis* (3, 5), of uniform size (8-10 cm in length) from Ría de Arosa (Galicia, Spain) were collected weekly and maintained in our laboratory with discontinuous feeding of a microalgal mixed in sea water aquariums at 15-16 °C.

Preparation of mantle tissue fragments.— About 20 mussels were used in each experiment. The mantle fragments were obtained as described (4).

Dose-response experiments.— Groups of fragments (30 mg) were incubated in glass tubes containing 0.5 ml of artificial sea water (ASW) with the following ionic composition in meq/l: Na^+, 512; K^+, 10; Cl^-, 500; Ca^{2+}, 5.2. The solution was buffered with 20 mM HEPES and 7.5 mM OHNa at pH 7.0. When required Ca^{2+} was replaced with the osmotically equivalent amount of NaCl.

Treatments were added in 100 µl of ASW or Ca^{2+}-free ASW (experiments with La^{3+} and EGTA), and final incubation volume adjusted to 1 ml with the same media.

Incubations were carried out at 30 °C for 120 min in a giratory shaker (150 cycles/min); and stopped at 4 °C by centrifugation (1.060 × g, 10 min). Glucose was determined in the supernatant by the glucose oxidase method (1) using the commercial diagnostic kit (Glucinet) from Sclavo (Siena, Italy).

Statistical calculations.— All results are expressed as the mean ± SEM together with the number of individual determinations (n). The statistical significance of differences between means was assessed by the Student's «t» test (17).

Results

Basal glucose releasing activity.— EGTA (0.1, 1 and 2 mM) in absence of external Ca^{2+}, showed an inhibition of the basal glucose release from mantle fragments, being maximun at 2 mM EGTA. Chlorpromazine (CP) in a µM range (50-200) in presence of external Ca^{2+}, also inhibited the basal glucose release (fig. 1).

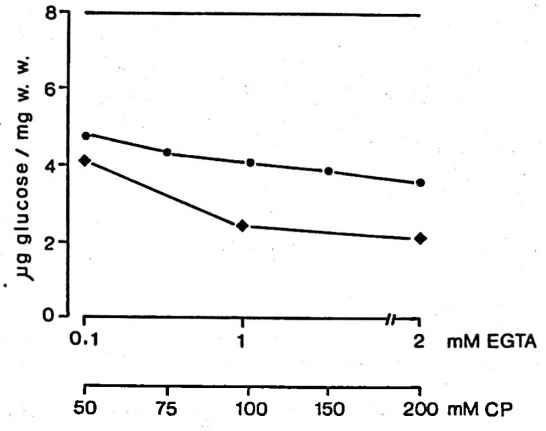

Fig. 1. *EGTA (◆) and chlorpromazine (CP) (●) dose-response in mantle tissue fragments.* Basal values are plotted above, and data in µg glucose/mg wet weight are expressed, as the arithmetic mean of individual determinations (n = 8), SEM = 4-6 %.

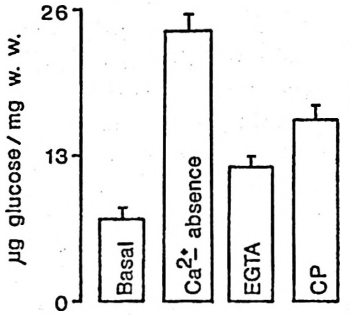

Fig. 2. *Inhibition on glucose release stimulation in external Ca^{2+} absence in mantle tissue fragments, by EGTA (2 mM) and CP (0.2 mM) compared with basal values.* Results in µg glucose/mg wet weight are expressed as the arithmetic mean of individual determinations (n = 10), SEM = 5-8 %.

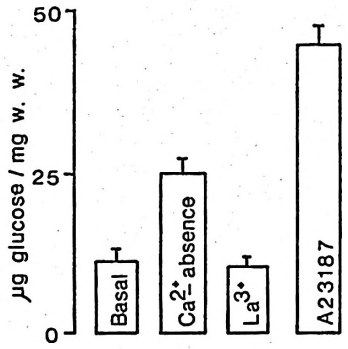

Fig. 3. *Stimulation of glucose releasing activity by A23187 (10 µM) compared with external Ca^{2+} absence stimulation, and Ca^{2+} absence glucose release reduction to basal values by La^{3+} (3 mM) presence.*
Results in µg glucose/mg wet weight are expressed as the arithmetic mean of individual determinations (n = 10), SEM = 5-8 %.

Stimulated glucose releasing activity.— The absence of external Ca^{2+} ions stimulated glucose release 2 to 3-fold over the basal value. Both, EGTA (2 mM) and CP (200 µM) decreased the glucose release promoted by absence of Ca^{2+}, but the inhibition obtained with EGTA was slightly higher that caused by CP (fig. 2).

Glucose release in absence of external Ca^{2+} increased with A23187 (10 µM), being this release the maximun obtained in the experiments. The presence of 3 mM La^{3+} decreased glucose release in Ca^{2+} absence to the basal values (fig. 3).

Discussion

Until now, the knowledge of the glycogenolytic regulation in the mantle tissue of molluscs has come from studies which only explain the regulation of glycogenolytic enzymatic cascade, the glycogenolytic activity in the mantle being understood as a source of glucose intented to be used intracellularly, through the glycolysis pathway (6, 9, 12). Nevertheless, in this paper, a fraction of the glycogenolytic activity in the mantle yielding glucose that is transported from the glycogen cells to other areas where this monosaccharide is needed in other metabolic pathways, is studied.

Thus, the results reported show a basal glucose release in mantle tissue, a fraction of which is inhibited by EGTA and CP. Stimulated glucose release is observed in external Ca^{2+} absence, which is also reduced by EGTA and CP. The Ca^{2+}-absence stimulated glucose release is increased by A23187. It follows from these facts: a) Ca^{2+} ions, and perhaps calmodulin, and b) Ca^{2+} ions might act as a triggering signal, i.e. whenever intracellular cytoplasmatic free-Ca^{2+} levels augment over the basal value, the glycogenolytic cascade is turned on.

Glucose release stimulation from mantle by external Ca^{2+}-absence can be due to a disorder in cellular Ca^{2+}-homeostasis when external Ca^{2+} ions are not present and it could cause a rise on cytoplasmic Ca^{2+} from intracellular stores which is also increased in the presence of A23187. The effect of EGTA on glucose release stimulation in absence of external Ca^{2+} would be explained by the fact that EGTA does not allow a rise in the intracellular Ca^{2+}, because of its fast efflux from the cells.

From the present results, the reduction to basal values of the Ca^{2+}-absence stimulated glucose release by lanthanum, would be expected, as La^{3+} does not allow the cellular Ca^{2+} influx-efflux movements (10) so that the intracellular cytoplasmatic Ca^{2+} ions are maintained at the basal values.

In conclusion, mantle tissue seems to have a glucose releasing activity that can support the existence of intercellular glucose transference. This glucose might originate from sources that generate glycogenolytic activity that in turn might be regulated by intracellular Ca^{2+} ions.

Acknowledgements.

This work was supported by grants from «Dirección General de Ordenación Universitaria y Política Científica» and «Consellería de Pesca. Xunta de Galicia» (Spain), 1988.

Resumen

Se estudia la actividad liberadora de glucosa en el tejido del manto de *Mytilus galloprovincialis*. El tejido del manto presenta una actividad basal de liberación de glucosa. La ausencia de Ca^{2+} externo aumenta de 2 a 3 veces la liberación basal de glucosa, y la presencia simultánea de A23187 (10 µM) dobla la liberación obtenida en ausencia de Ca^{2+} externo. EGTA (2 mM), clorpromazina (200 µM) y lantano (3 mM) disminuyen la liberación de glucosa promovida por la ausencia de Ca^{2+} externo. Estos resultados sugieren que la actividad liberadora de glucosa en el tejido del manto podría estar regulada por los iones Ca^{2+}.

Palabras clave: *Mytilus galloprovincialis*, Liberación de glucosa, Iones calcio.

References

1. Bergmeyer, H. U. and Bernt, E.: In «Methods of Enzymatic Analysis» (H. U. Bergmeyer, ed.). Academic Press, Inc., New York, 1974, pp. 123-130.
2. Cohen, P.: In «Calcium and Cell Function» (Cheung, W. Y., ed.). Academic Press, Inc., New York, 1980, 1.
3. Crespo, C. A.: In «Histofisiología de las reservas bioenergéticas del manto de *Mytilus galloprovincialis* Lmk.». Doctoral These. Facultad de Farmacia. Santiago de Compostela, 1989.
4. Crespo, C. A., García-Caballero, T., Beiras, A. and Espinosa, J.: *J. Moll. Stu.*, 56, 127-128, 1990.
5. Crespo, C. A. and Espinosa, J.: *Rev. esp. Fisiol.*, 45, 117-122, 1989.
6. De Zwaan, A.: In «The Mollusca» (K. M. Wilbur, ed.), Academic Press, Inc., New York, 1983, 1, pp. 173-175.
7. De Zwaan, A. and Zandee, D. I.: *Comp. Biochem Physiol.*, 43 A, 53-58, 1972.
8. Exton, J. H.: *Clin. Neuropharmacol.*, 9, 54-58, 1986.
9. Gabbott, P. A.: In «The Mollusca» (K. M. Wilbur, ed.), Academic Press, Inc., New York, 1983, 2, pp. 196-201.
10. Gabbott, P. A., Whittle, M. A., Head, E. J. H. and Runham, N. W.: *Eur. Soc. Comp. Biochem. Physiol. Meet.* (3rd), Amsterdam, Pergamon Press, Oxford, 1981, pp. 109-110 (abst.).
11. Hellman, B.: *Biochem. Biophys. Acta*, 540, 534-542, 1978.
12. Hemminga, M. A., Maarkant, J. J., Koowen, W. and Joosse, J.: *Gen. Comp. Endocrinol.*, 57, 117-123, 1985.
13. Lubet, P., Herlin, G., Mathieu, M. and Collin, F.: *Haliotis*, 7, 59-62, 1976.
14. Lubet, P. and Mathieu, M.: *Malacologia*, 22, 131-136, 1982.
15. Mathieu, M.: *J. Exp. Zool.*, 241, 247-252, 1987.
16. Sokal, R. R. and Rolf, F. J.: Biometry. Freeman, San Francisco, 1975.
17. Studer, R., Swowdowne, K. and Borle, A.: *J. Biol. Chem.*, 259, 3596-3604, 1984.