Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Effects of 5-cystein-S-yldopa on the development of sea urchin eggs

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **74** (1983), n.2, p. 93–98.

Accademia Nazionale dei Lincei

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1983.

Biochimica. — *Effects of 5-cystein-S-yldopa on the development of sea urchin eggs.* Nota di BERNDT HAGSTRÖM (*), GIOVANNA MISURACA (**), ANNA PALUMBO (*) e GIUSEPPE PROTA (***), presentata (****) dal Corrisp. A. RUFFO.

RIASSUNTO. — In relazione alle ricerche sull'attività biologica della 5–S–cisteinildopa, è stato esaminato l'effetto di questo amminoacido fenolico sullo sviluppo embrionale dell'uovo del riccio di mare *Paracentrotus lividus*. Ripetuti esperimenti hanno mostrato che tale composto, anche a concentrazione di 10^{-3} M, non presenta alcuna tossicità nei confronti del sistema da noi considerato. Al contrario, in una serie di osservazioni parallele, è stato possibile evidenziare un effetto positivo della 5–S–cisteinildopa sia sulla fertilizzazione che nei successivi stadi dello sviluppo embrionale.

5-cystein-S-yldopa (cysdopa) is a naturally occurring catechol aminoacid (Prota, 1980) which arises biogenetically by addition of cysteine or glutathione to dopaquinone derived by enzymic oxidation of tyrosine and/or dopa. It was originally described as a key intermediate in the biosynthesis of phaeomelanic pigments (Prota and Thomson, 1976); but it is now clear that cysdopa is normally found in blood and urine (Hansson *et al.*, 1979; Rorsman *et al.*, 1979). In more recent years, evidence has been obtained that cysdopa and related metabolites are more widely distributed in nature than it was previously believed, occurring also in birds (Agrup *et al.*, 1979), fishes (Ito and Nicol, 1977) and even in some marine invertebrates (Carlberg *et al.*, 1982), e.g. sea anemones and crustaceans. Such a wide distribution would suggest a more fundamental role of cysteinyldopas in biological systems rather than of, or in addition to, intermediate metabolites of melanogenesis.

However, up to the present, little is known about the biological properties of these metabolites, except a study by Fujita *et al.* (1980) on the effect of cysdopa on the growth of several human tumor cell lines. In this it was found that the aminoacid is selectively toxic to malignant cells and is also active against murine M1210 leukemia and B–16 melanoma *in vivo* with no untoward effects on the host. These results prompted us to study the effect of cysdopa on the develop-

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(****) Nella seduta del 12 febbraio 1983.

ment of sea urchin eggs which provides a most convenient system for testing the biological activity of substances on both cell cleavage and differentiation (Hagström and Lönning, 1973).

Materials and methods

Cysdopa was obtained by enzymic co-oxidation of L-dopa and L-cysteine according to the procedure described by Ito and Prota (1977). A stock solution of cysdopa was prepared in 0.45 M aqueous NaCl and adjusted to pH 7.5 or 8.1 immediately before use. Solutions of cysdopa at 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M were obtained by adding suitable amounts of the stock solution to sea water containing sea urchin eggs. The stability of the product under the testing conditions was followed both spectrophotometrically and by chromatography on a Liquimat III (Kontron, Switzerland) amino acid analyzer using pico C lithium buffer (Durrum) at 70 °C as eluent.

All the experiments were carried out with gametes and embryos from the sea urchin species *Paracentrotus lividus* (Lamarck). In each experiment only one female and one male were used in order to ensure a material as homogeneous as possible. The gametes were obtained by opening the animals, and fertilization was carried out at a concentration of 2000 eggs per ml and 10^5 spermatozoa per ml. The temperature of the experiments was kept at 18–19 °C.

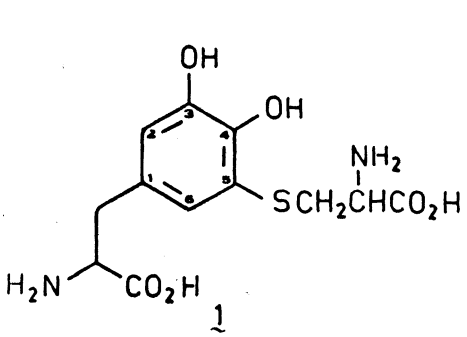
The effect of the test substance was assessed in fertilization rate experiments, where the sperm-egg interaction is stopped 5, 10, 15, 20 etc. seconds after insemination (zero time) by the addition of sodium lauryl sulphate. The infinity point (∞) is used for the study of cleavage and differentiation up to the pluteus stage, about 48 hours after insemination. The substance was added at insemination or at a defined time after insemination; the substance was then removed after two hours of treatment by repeated washings in pure sea water. The concentration of cysdopa ranged from 10^{-6} to 10^{-3} M.

In the Gulf of Naples the sea water has a pH of 8.1, which is a little above the optimum pH-range of stability of cysdopa. We therefore made parallel experiments at pH 7.0–8.1 including controls at the lower pH. Fertilization and development are influenced negatively by a pH below the normal pH 8.1.

Results and discussion

Previously, we have found (Palumbo *et al.*, 1982) that in the presence of bivalent cations including Ca^{++}, cysdopa (**1**) undergoes spontaneous oxidation to give a cyclisation product, identified as the 1,4-benzothiazine acid **2**. Consequently, the stability of cysdopa in sea water under the testing conditions was

preliminarly investigated by U.V. spectroscopy and by cromatography on amino acid analyzer.



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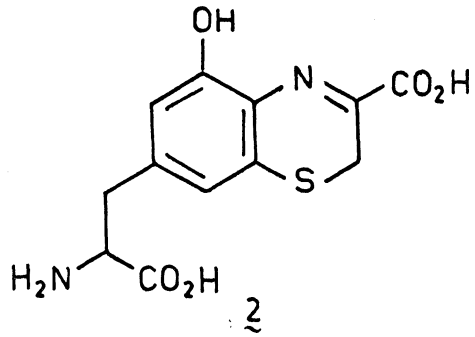

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As can be seen from data reported in Table 1, over a period of about two hours the oxidation is almost negligible, although the rate of reaction becomes significant at concentrations as high as 10^{-3}. As expected, a considerable slow down of the rate of oxidation is observed when cysdopa is dissolved in sea water adjusted to pH 7.5 whereby even at concentration of 10^{-3} the product remains practically unchanged after two hours. Under the same conditions L–β–3,4-dihydroxyphenyl alanine (DOPA) is rapidly oxidized thus making it a difficult parallel study of the biological activity of this related catechol aminoacid.

TABLE 1

The effect of pH on the stability of cysdopa in sea water at different concentrations.

Cysdopa concentration	pH	% of unchanged cysdopa after		
		2 hr	4 hr	7 hr
$0.56 \cdot 10^{-3}$ M	7.5	99.5	96	84.5
	8.0	97	88	68.6
$1.1 \cdot 10^{-4}$ M	7.5	100	97	95
	8.0	99.3	92	84

With this background available, the effects of cysdopa on the development of sea urchin eggs was then investigated. Preliminary experiments indicated that the substance had a general positive effect on fertilization, cleavage and differentiation. Only in the highest concentrations tested i.e. above 10^{-4} M, was there a slight retarding effect on fertilization, though 100% fertilized eggs was always obtained.

The fact that cysdopa acted positively and had almost no toxic effects in this test system, imposed the main difficulty in this investigation. When using a perfectly normal biological material, and in this respect *Paracentrotus* of the Gulf of Naples is outstanding, it became difficult to observe a superimposed positive effect. Therefore, the sensitive fertilization rate method proved to be helpful in this context. Moreover, we found that suboptimal gametes presented the best material for assessing the positive effects of cysdopa.

Since the pH suitable for *Paracentrotus* (pH 8.1) does not coincide with the optimum pH range of stability of cysdopa, we made simultaneous experiments with the same biological material at different pH. A typical experiment is shown in Table 2. The concentrations of cysdopa ranged between 10^{-6} and 10^{-4} and two parallel series were made with gametes from the same animals at pH 7.5 and 8.1. The substance was present at insemination and was removed by washings in normal sea water 2 hours after fertilization. Under these conditions no appreciable oxidation or degradation of cysdopa took place during the short period of treatment (cf. Table 1). The results registered in Fig. 1 are therefore due to the interaction with the substance during the two hours of exposure. Those from Table 2 (nos 1 and 5) show that the fertilization rate becomes somewhat retarded at pH 7.5. It is also evident that cysdopa acts more positively at pH 7.5 than at pH 8.1.

TABLE 2.

Fertilization rate experiment. Eggs and sperm from Paracentrotus lividus. Cysdopa was added at the moment of insemination. Each percentage quoted is based on counts of about 250 embryos.

	% fertilized eggs seconds after insemination					
	5	10	15	20	30	∞
1) Control, pH 7.5	11	54	93	96	98	100
2) Cysdopa 10^{-4} M, pH 7.5	5	60	87	90	96	100
3) Cysdopa 10^{-5} M, pH 7.5	36	80	94	99	100	
4) Cysdopa 10^{-6} M, pH 7.5	21	87	95	97	99	100
5) Control, pH 8.1	31	87	93	100		
6) Cysdopa 10^{-4} M, pH 8.1	19	72	92	98	100	
7) Cysdopa 10^{-5} M, pH 8.1	25	86	97	100		
8) Cysdopa 10^{-6} M, pH 8.1	59	86	97	98	100	

During the cleavage stage, from 2-cell to about 250 cells, there was a general improvement both in the rate of cleavage and the morphological appearance of

the embryos following treatment with cysdopa (Fig. 1). It is important that this improvement was manifest at both pH 7.5 and 8.1. Moreover the positive effect became accentuated at the high concentration of 10^{-4} M of cysdopa. At the 4-cell stage since after two hours of treatment the substance was removed, the later effects on differentiation result from the short treatment with cysdopa. In the ensuing development the positive effects of cysdopa became still more apparent. 45 hours after insemination (i.e. 43 hours after the substance was removed) the controls were in an early pluteus stage. The embryos which had been treated for two hours were all in a far more advanced stage of differentiation (cf. Fig. 1, *a-d*). However, it is not only the stage of development which is improved. The size of the larvae has also become substantially increased, which is seen from a comparison of the controls (Fig. 1 *a* and *c*) with cysdopa treated larvae (Fig. 1*b* and *d*).

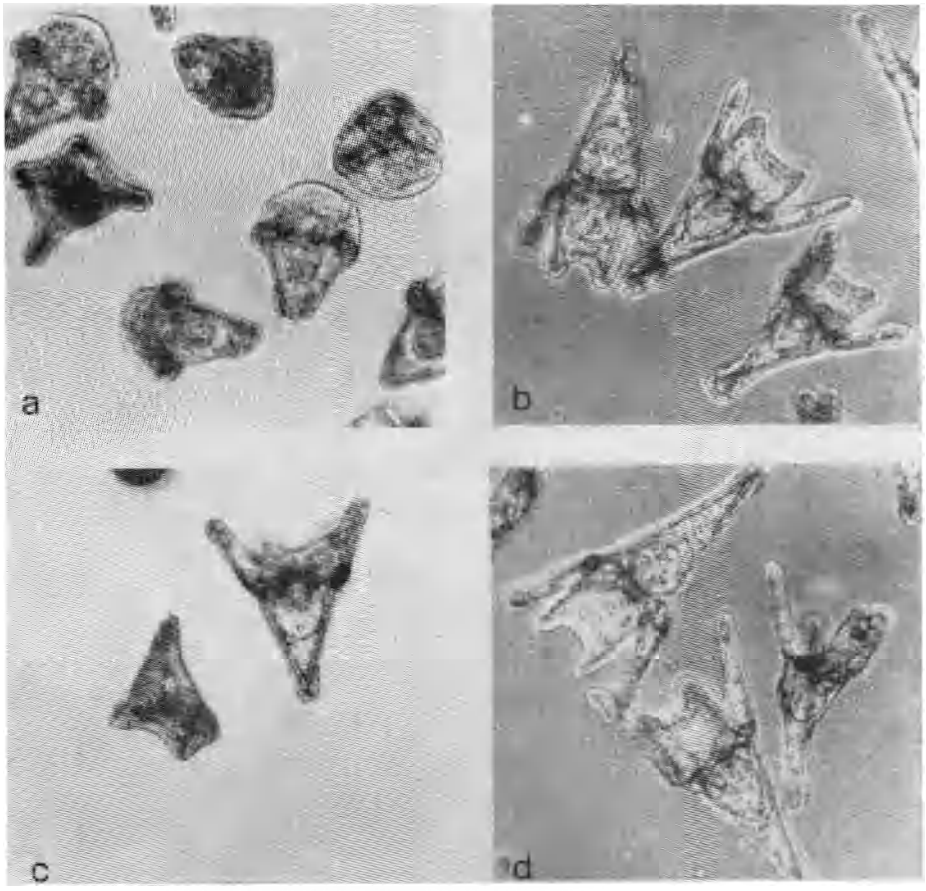

Fig. 1. – Effects of cysdopa on the early development of sea urchin eggs *a*: control at pH 7.5, prism-early pluteus stage. *b*: treatment in 10^{-4} M cysdopa for 2 hours from insemination, well-developed plutei pH 7.5. *c*: control at pH 8.1, early pluteus stage. *d*: treatment in 10^{-4} M of cysdopa for 2 hours from insemination, advanced plutei, pH 8.1. Magnification: 150×.

The results of our experiments indicate that cysdopa in concentration up to 10^{-4} M acts positively on the differentiation of the sea urchin embryo. The toxicity of the substance is low since 100% fertilization is obtained even in the presence of 10^{-3} M of cysdopa.

At present it seems difficult to make any definitive conclusion about the mechanism of action of cysdopa. However, the fact that cleavage becomes promoted by a short treatment with cysdopa would suggest an activation of some enzyme system(s) operative in the egg surface during fertilization. We are currently investigating whether the reported cytotoxicity of cysdopa to malignant cells is actually related to differences in the basic metabolism between normal and tumoral cells or rather to differences in the experimental conditions used for testing the activity of cysdopa on cell growth.

Acknowledgement. This work was supported by grants from M. P. I and C.N.R. (P.F. Controllo della crescita neoplatica) Roma Contratto N. 81.01458.96. The technical assistence of Mrs. A. Criscuolo was greatly appreciated.

References

AGRUP G., HANSSON H., RORSMAN H., ROSENGREN A. M. and ROSENGREN E. (1978) – « Acta Derm. Venereol. (Stock.) », *58*, 269.

CALBERG M., LAXMYR L., ROSENGREN E. and ELOFSSON R. (1982) – « Comp. Biochem. Physiol. », *73c*, 23.

FUJITA K., ITO S., INOUE S., YAMAMOTO Y., TAKEUCHI J., SHAMOTO M. and NAGATSU T. (1980) – « Cancer Research », *40*, 2543.

HAGSTRÖM B. E. and LONNING S. (1973) – « Acta Pharmacol. Toxicol. », *32*, 1.

HANSSON C., AGRUP G., RORSMAN H., ROSENGREN A. M., ROSENGREN E. and EDHOLM L. E. (1979) – « J. Chromatogr. », *162*, 7.

ITO S. and NICOL J. A. C. (1977) – « Biochem. J. », *161*, 499.

ITO S. and PROTA G. (1977) – « Experientia », *33*, 1118.

PALUMBO A., NARDI G., D'ISCHIA M., MISURACA G. and PROTA G. (1982) – in press on Gen. Pharmacol.

PROTA G. and THOMSON R. H. (1976) – « Endeavour », *35*, 32.

PROTA G. (1980) – In: *Natural Sulfur compunds*, p. 391. Eds D. Cavallini, G. E. GAULL, V. Zappia, Plenum Press, New York.

RORSMAN H., AGRUP G., HANSSON C., ROSENGREN A. M. and ROSENGREN R. (1979) – In: *Pigment Cell*, vol. 4, p. 244. Ed. S. Klaus, Basel, Karger.