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Dopamine, noradrenaline and serotonin during hypo-osmotic stress of *Carcinus maenas*

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Abstract

Dopamine, noradrenaline and serotonin were measured in the haemolymph and gills of *Carcinus maenas* before and after transferring them into diluted seawater. A remarkable variation of the above-mentioned compounds was observed as a function of time of osmotic stress. *C. maenas* were captured in the Venetian lagoon near Chioggia, Italy in 1986.

Introduction

It has been reported that dopamine (DA) may be implicated as a neurohormone in crustacea, but its physiological role has not yet been clarified (Baker and Hooper, 1975; Atwood, 1984). The release of DA has been demonstrated to occur near the cell bodies of the neurosecretory neurons, in the pericardial organs (PO) close to the heart. In this way a rapid distribution from the heart to the haemolymph and the gills, as well as to the other parts of the body, is ensured. PO being the largest neurosecretory organs of crustacea, they were hypothesized as being the site for the production and release of biogenic amines (Baker and Hooper, 1975). PO are neurohemal structures specialized in the production and storage of catecholamines, phenolamines and peptides for release into general circulation, probably as neurohormones.

Cook and Sullivan (1983) reported that, in the Macruran, octopamine is much more evident than DA, while the opposite is true in the Brachyura. Some authors (Baker and Hooper, 1975) have shown that the major tyrosine metabolite is stored in the PO of *Carcinus maenas, Cancer* sp., *Cardiosoma* sp., which supports the hypothesis that DA may be a neurohormone. According to Kamemoto and Oyama (1982), homogenates of PO increase Na^+ flux of the perfused gills of *Carcinus* sp. and *Callinectes* sp., also increasing cAMP levels of the gill tissue. DA can also act as a neurohormone or a neurotransmitter in the stomatogastric ganglia as reported by Osborne and Dando (1970). Berlind (1977) suggested that PO participate in an integrated osmoregulatory function involving Na^+ transport, control of the rate of the haemolymph movement through the gills by means of the heart rate modulation and rate of water movement externally over the gills by influencing the scaphognatite.

Serotonin (5 HTP) is released into the ventral sinus and dorsal pericardial cavity by PO. It has been suggested by Sullivan and co-workers (Sullivan *et al.*, 1976, 1977; Sullivan, 1978) that 5 HTP, after being released from the PO, may play the role of a circulating neurohormone of widespread importance.

Materials and methods

Carcinus maenas were captured in the Venetian lagoon near Chioggia, Italy in 1986 and rapidly transferred to the laboratory into well aerated aquaria and kept at 15 °C. Individuals were starved for 2 d before experimentation.

Catecholamine determinations

Haemolymph was extracted from living individuals by puncturing the lower part of the carapace, and filtered through a cheesecloth. Gills were homogenized in $HClO_4$, serotonin, dopamine and noradrenaline were determined fluorimetrically by following the method reported by Kent Shellenberger and Gordon (1971). Experiments were repeated five times in ten different individuals.

Results and discussion

Dopamine and noradrenaline

When *Carcinus maenas* is transferred from normal seawater to 50% seawater (SW), a dramatic increase in

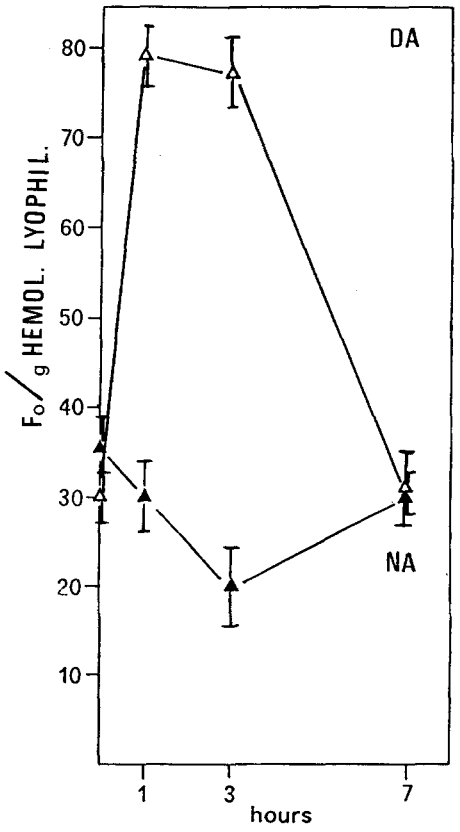

Fig. 1. *Carcinus maenas.* Dopamine and noradrenaline in the hemolymph as a function of hypo-osmotic stress time. Fo/g represents the fluorescence per gram of hemolymph lyophilized. Dopamine λ_{exc}: 345 nm, λ_{emiss}: 410 nm. Noradrenaline λ_{exc}: 285 nm, λ_{emiss}: 325 nm. (Bars: ± SD). Experiments were repeated five times in ten different individuals. △: dopamine; ▲: noradrenaline)

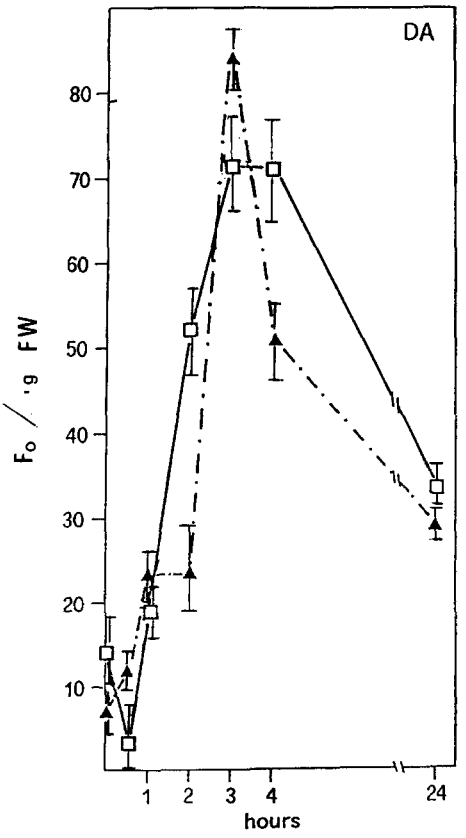

Fig. 2. *Carcinus maenas.* Dopamine extracted from gills of *C. maenas* transferred into 50% seawater. Data represent the fluorescence per g of fresh tissues as function of stress time (λ_{exc}, λ_{emiss} as in Fig. 1). (Bars: ± SD) (▲: anterior gills; □: posterior gills). Experiments were repeated five times in ten different individuals

dopamine occurs. In the hemolymph (Fig. 1), DA more than doubles while noradrenaline decreases by almost 50% with respect to the initial value. The maximal effect is registered between 1 and 3 h after the hypo-osmotic stress. In the gills (Figs. 2, 3) DA and NA show a similar behaviour, increasing respectively seven and three times with respect to the initial value. Little is known about the role of the catecholamines and in particular of DA as a neurohormone in crustacea, although several authors have suggested the possibility of an involvement in the osmoregulation of crustacea (Kamemoto and Oyama, 1982). The significant variation of catecholamine reported here further supports the hypothesis of the Japanese authors.

Serotonin

The rapid decrease of serotonin after transferring individuals to diluted SW is also significant. Fig. 4 describes the effect of hypo-osmotic stress on the concentration of 5HTP in the anterior and posterior gills of *Carcinus maenas.* While in the anterior gills there is a rapid decrease of 5HTP in the diluted medium and a constancy when a low level is reached, in the posterior gills, 5HTP rapidly decreases between 1 and 3 h and 7 h after the stress is again found at the initial concentration. 5HTP has been reported to stimulate adenylate cyclase in the gills of different species of mollusca, suggesting a possible control of 5HTP on Na^+ flux (Scheide and Dietz, 1984). Livingston *et al.* (1981) reported a Na^+ dependent, high affinity uptake of serotonin in the lobster nerve. 5HTP can increase the rate of scaphognatite movement and consequently can increase the water circulation (Berlind, 1977), thus promoting a more rapid oxygen exchange in the tissues, as a consequence of increased oxygen demand due to the hypo-osmotic conditions. Furthermore, 5HTP stimulate fluid secretion in the malpighian tubules of locusta (Morgan and Mordue, 1984). It is known that catecholamines can act in different ways in fish, provoking phenomena such as an increased rate of water and urea transport in the gills, an increased transfer of oxygen in the tissue, affecting vascularization and membrane permeability etc. There is some indication of a possible relationship between catecholamines, serotonin and hypo-osmotic stress, but much more work must be carried out to clarify the involvement of these compounds on the osmoregulation of crustacea.

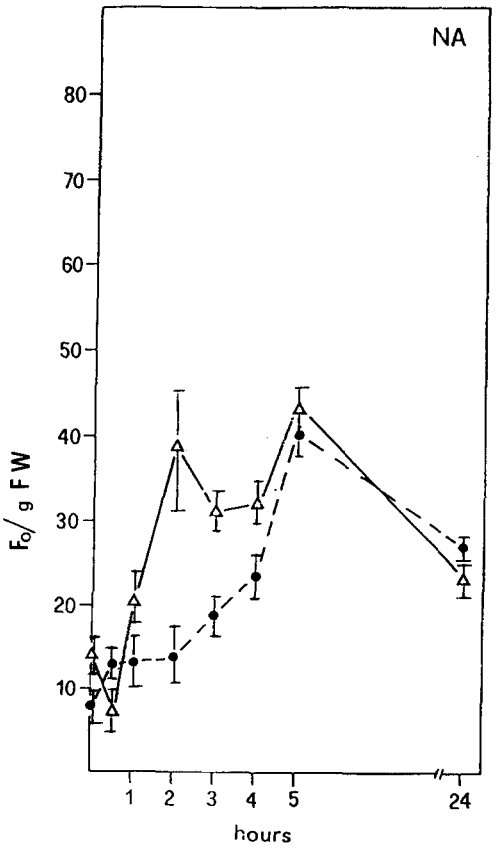

Fig. 3. *Carcinus maenas.* Noradrenaline extracted from gills of *C. maenas* transferred into 50% seawater. Data report the fluorescence per g of fresh tissue as function of stress time (λ_{exc}, λ_{emiss} as in Fig. 1). (Bars: ± SD) (●: anterior gills; △: posterior gills)

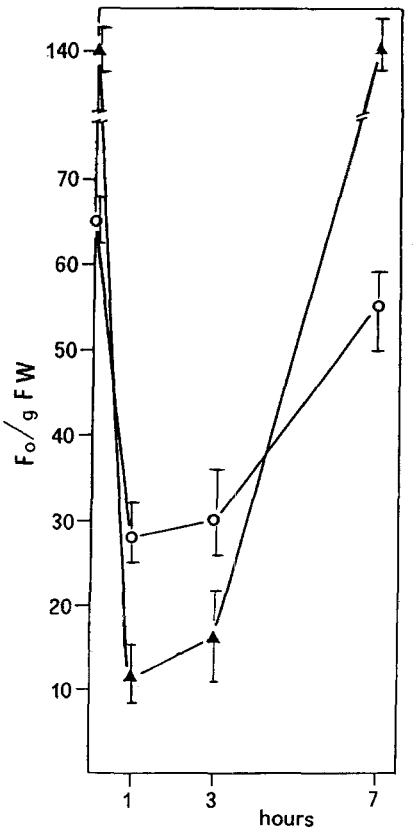

Fig. 4. *Carcinus maenas.* Serotonin extracted from gills of *C. maenas* after transferral into 50% seawater. Data report the fluorescence per g of fresh tissue as function of stress time (λ_{exc}: 295 nm, λ_{emiss}: 340 nm). (Bars: ± SD) (○: anterior gills; ▲: posterior gills)

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