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EFFECTS OF BIOGENIC AMINES ON THE REGENERATION OF SMALL PIECES OF THE PEDAL DISC OF THE SEA ANEMONE *METRIDI UM SENILE* (LINNAEUS)

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Abstract-1. The effects of the biogenic amines adrenalin and 5-hydroxytryptamine and of their respective precursors DOPA and 5-hydroxytryptophan on the regeneration of small pieces of the pedal disc of the sea anemone *Metridium senile* are analysed.

2. The biogenic amines with a phenolic nucleus had a promotive effect while any eventual promotive effect of the indole alkylamines was disturbed by their toxicity.

3. These results and conclusions are compared with those obtained from similar works on animals from other invertebrate Phyla.

INTRODUCTION

The sea anemone *Metridium senile* has a large capacity for regeneration. Small pieces (3 x 3 mm) of the pedal disc isolated from the animals can give rise to complete new polyps within a few weeks. This property seems linked to its mode of reproduction by laceration of the foot (Polteva & Lenicque, 1970). In previous studies on the mechanism of regeneration of small pieces of the planarian worm *Dugesia tigrina* it was shown that the biogenic amines 5-hydroxytryptamine (5-HT), adrenalin (NA), and their respective precursors 5-HTP and DOPA, have a promotive role in the control of regeneration (Lenicque & Jacobson, 1969; Lenicque, 1971, 1974; Lenicque & Feral, 1976). It is known that sea anemones synthesize the indole alkylamine 5-HT in addition to biogenic amines with a phenolic nucleus, such as DOPA and dopamine. The aim of this work is to investigate whether or not the biogenic amines play a role in the regeneration processes of *Metridium senile* as well as those of *Dugesia tigrina*. If so, this mechanism must be a very primitive one because sea anemones and planarian worms belong to different phyla.

MATERIAL AND METHODS

Individuals of *M. senile* were collected in the Gullmar fjord at the Kristeneberg Marine biological station (Fiskebäckskil, Sweden). The pedal disc was isolated from the body with small scissors and iris-scalpels it was cut into small pieces containing 3-4 chambers. Mucus was removed with a pipette. These pieces were allowed to regenerate in sea water at approx 11°C. The progress of regeneration was observed every day during 3 weeks. The test of regeneration was the formation of the column, at which stage the animals have the shape of a pear. There were about 50 pieces of animal in every treated population and control group. The experiments were repeated 3 times or more, giving a minimum of 150 animals for every concentration of substance tested. The results were considered as highly significant when $P < 0.01$.

The substances were dissolved in sea water. Treatments were performed overnight and during the day the regenerating animals were cultivated in running sea-water. This procedure was continued for a week. The biogenic amines were 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), DOPA and adrenalin (NA). After the preliminary experiments, the following concentrations were applied: 5-HTP and 5-HT at 5 MIO^{-}'; 9 MlO^{-}'; 2.5 M1O"'; DOPA and NA at 5 M1O^{-6}; 25 M1O^{-6}; 5 MlO^{-5}. Para chlorophenylalanine (pCPA), an inhibitor of hydroxylation of tryptophan, was used at saturation. Lithium chloride was used at 2 M1O^{-}' 3 M1O^{-3}; 3.5 M1O^{-3}. The pH was adjusted to 7.5 when necessary. The biogenic amines and their precursors were purchased from Sigma (St. Louis, U.S.A.) and pCPA from Kistner (Stockholm, Sweden).

Some animals were fixed and stained with Alcyan blue at different pH values to observe the mucopolysaccharides, and others were stained with Mallory's mixture for the observation of collagen. The enzymatic activities of monoamine oxydase (MAO) and alcalin phosphatase were detected by the methods of Koelle & Glenner, and Gomori respectively (Pearse, 1972).

RESULTS

As the development of small pieces of the pedal disc of *Metridium senile is* not a classical tool in experimental embryology, a table was determined for the stages of regeneration. Five steps could be distinguished (Fig. 1). (1) Healing: 24 hr after the operation., The tissues of the ectoderm and endoderm came into contact and the edges of the two wounds fused. The cells of the ectoderm were normal and they secreted large quantities of mucus. Dedifferentiation began in the endoderm and in the mesoglea. (2) Disintegration of the structures of the animals: this stage lasted several days, varying in duration according to the temperature and oxygenation of the water. The mesentheres disappeared. The mesoglea was broken up. At this stage the small pieces had the appearance of turgescent spheres. They adhered to the bottom

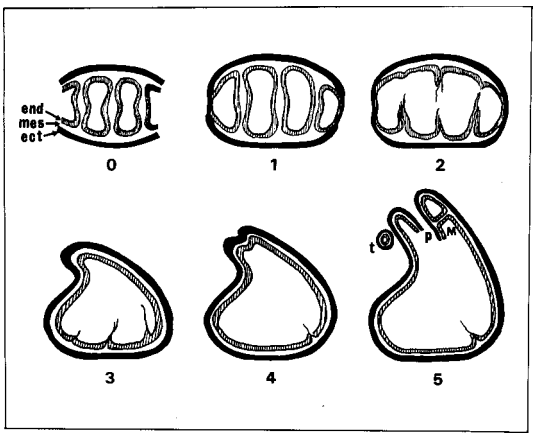

Fig. 1. Table of development of the pieces of pedal disc of *Metridium senile* (Linnaeus). "O". Immediately after the operation. I. Healing band of ectodermal and endodermal cells. At this stage the animal is spherical and turgescent. II. Degradation of the tissues, mostly the' mesentheres. III. Formation of the column. Note the kind of blastem of regeneration at the top of the animal. IV. Formation of the pharynx by invagination of the tissues at the top of the column. V. Formation of new tentacles and mesentheres (P = pharynx, t = tentacle in transverse section, M = new mesenthere).

of the Petri dishes. (3) Formation of the column: the animal became pear-shaped and was characterized by a high alkaline phosphatase activity in the new endoderm at its tip. Numerous mitoses were observed in the ectoderm which secreted mucus at a normal rate. A new mesoglea was synthetized between the ectoderm and the endoderm. (4) Invagination of the pharynx. (5) Formation of new mesentheres and new tentacles.

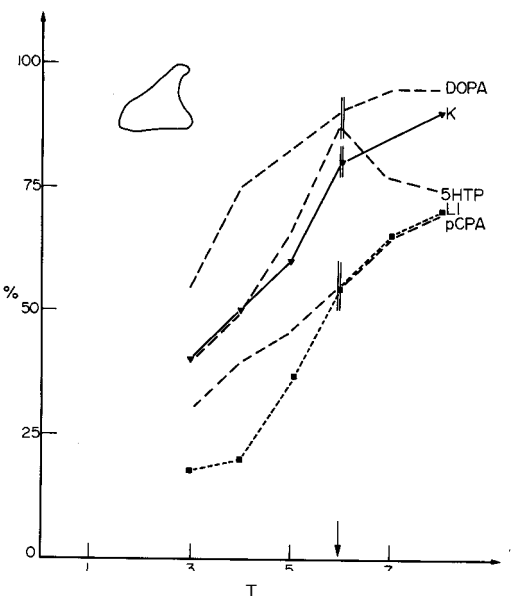

Fig. 2. Effects of 1 DOPA, 5-hydroxytryptophan (5-HTP), Lithium chloride (Li), and p chlorophenylalanine (pCPA) on the rate of regeneration of small pieces of the pedal disc. Control = K. x axis: percentage of animals at stage III. y axis: time in days. The promotive effect of I DOPA was highly significant ($P < 0.01$).

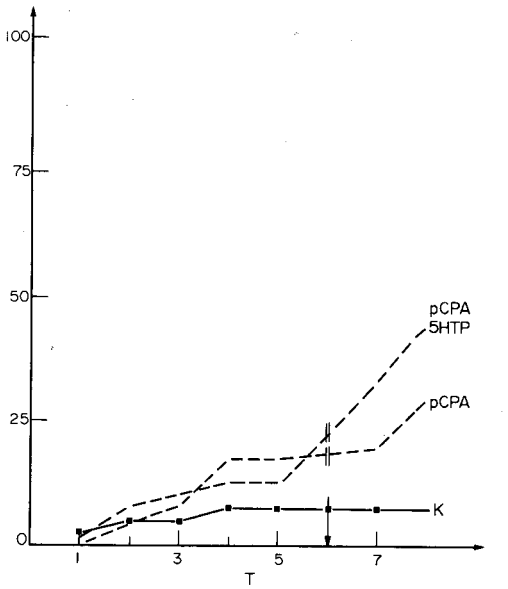

Fig. 3. Toxic effect of pCPA; addition of 5-HTP had no protective action. x axis: percentage of dead animals. y axis time in days. Controls = K.

Stage 3, the formation of the column, was taken as a test of development. It was possible to observe that the biogenic amines NA and DOPA, at the doses of 5 MlO^{-6} to 5 MlO had a promotive effect on regeneration. The treated populations reached stage 3 before the control groups. The differences in the rates of development were highly significant ($P < 0.01$) (Fig. 2). In contrast it was not possible to detect a similar promotive effect when the treatments were performed with 5-HTP or 5-HT because the results were affected by a toxic effect leading to the death of many animals about a week after the operation (Fig. 3). pCPA had an inhibitive or toxic

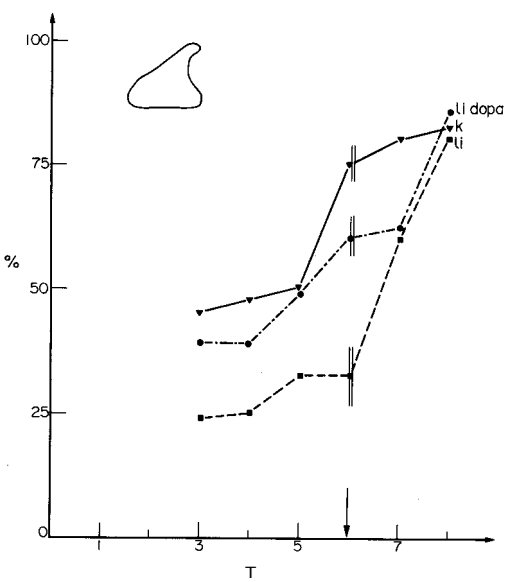

Fig. 4. Protective effect of 1 DOPA against the inhibitive effect of C1Li. This effect is highly significant ($P < 0.01$). Lithium chloride (Li); Lithium chloride and DOPA (LiDOPA), control = K. x axis: animals at stage III; y axis: time in days.

effect at the dose of M10'. Lithium chloride had the same effect at 2 to 3 $M10^{-3}$ (Fig. 2). DOPA had the effect of protecting the animals against the toxic effects of LiC1. The differences in the rates of development with LiCl + DOPA and LiCI alone were highly significant on days 3-6 ($P < 0.01$) (Fig. 4). 5-HTP did not have any protective effect on pCPA, but on the contrary, it enhanced the toxic effect of the latter.

DISCUSSION

Biogenic amines have been proposed to be of importance in differentiation following analytical and behavioural studies of gastropod embryos (Koshtoyants *et al.,* 1961), sea urchin embryos (Buznikov *et al.,* 1964, 1972; Gustafson *et al.,* 1970, 1972, 1973) and polychaete embryos (Emanuelson, 1974). It has also been suggested that monoamines are of importance in the regeneration of Planaria (Lenicque & Jacobson, 1969; Lenicque 1971, 1974) and oligochaetes (Chapron, 1972). It was of interest therefore to test their effects on animals, Anthozoa belonging to another phylum. The biogenic amines seem to play a role of promotive agents during the development of all three systems, but there are some differences. Probably, the natural hormone for developing sea urchin embryos and planarian worms is 5-HT, whereas regenerating oligochaetes and sea anemones seem to be under the control of a biogenic amine with a phenolic nucleus. The present work has shown that both 5-HTP and 5-HT are toxic to *M. senile* despite the fact that these substances, as well as DOPA, dopamine and NA, could be detected in these animals by biochemical analysis (Lenicque *et al.,* 1976). It may be that 5-HT does play a physiological role in regeneration of small pieces of the pedal disc, but only during very short periods or flashes. In which cellular mechanism does DOPA play a role when promoting regeneration in *M. senile?* Three main mechanisms will be investigated; the rate of mitosis, protein-synthesis, and cell-migration. Mitosis and protein synthesis, principally of collagen, appear to be very important in the blastem of regeneration during formation of the column. We have done a preliminary work on the influence of DOPA on the synthesis of collagen by means of autoradiography. It seems that the rate of incorporation of tritiated proline in the ectoderm is increased in pieces of animals treated with DOPA. Thus on the fourth day after the operation the numbers of grains of silver for the same unity of ectoderm surface was 49 for treated animals and 16 in the control groups ($P < 0.005$). This analysis will be completed in a few months.

CONCLUSION

It seems that the biogenic amines adrenalin (or its precursor DOPA) and perhaps 5-hydroxytryptamine (or its precursor 5-hydroxytryptophan) play a promotive role during regeneration of small pieces of the pedal disc of *M. senile.* This role must be a primitive and general one because it has also been detected in regenerating planarian worms and oligochaetes as well as in sea anemones.

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