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Hydrocortisone and Corticosterone in the Reproductive Organs of *Illex illecebrosus* (LeSueur, 1821)

by

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Abstract

A quantitative determination of glucocorticoids (hydrocorti- and - corticosterone) in the reproductive system organs of short-finned squid (132 females and 162 males) has been carried out to obtain the data on the variations of its content in different stages of the life cycle.

Steroids have been extracted with the aid of acetone and then with the aid of ether. Hormone purification has been carried out by the thin layer chromatography method in different systems.

Glucocorticoid concentration has been determined as μg/g of tissue in female gonads and nidamental glandules at stages of maturity of reproductive system II, III, as well as in male gonads and spermatophore sacs at stages of maturity of reproductive system II, III, IV, V. Maximum hormone concentration has been found in male spermatophore sacs at stage of maturity II and in female gonads at stage of maturity II. Minimum concentration has been observed in one male spermatophore sac at stage of maturity V.

A trend towards glucocorticoid concentration decreases when squid maturing has been revealed. At earlier stages of maturity concentration decrease is more pronounced in males than in females.

An assumption has been made as to the possible functional role of glucocorticoids in spermatogenesis as energy accumulation regulators. A possibility has been discussed as to glucocorticoid effect on neurohormonal action upon short-finned squid and other cephalopod molluscs reproductive systems.

Introduction

An initial information on the existence of steroid type substances in the invertebrates has been obtained in the fifties (Cenovic, 1954; Hagerman, Wellington, Villee, 1957; Dancasiu, Istrati, 1958; Lisk, 1961), and only by 1970 a problem of steroidogenesis existence in the tissues of a number of invertebrates has been resolved in a positive manner (Tcholakian, Eik-Nes, 1969; Lenoux, Sandor, 1970; Teshima, Kanazawa, 1970; Teshima, Kanazawa, 1971; Lupo di Prisco, Fulgheri, 1975). However, there are no data on steroid quantitative distribution and their role in different organs of invertebrates. The existence of high-specific albumin receptors of steroid hormones in the cells of very different tissues of the vertebrate animals points out to a high functional activity of steroid hormones (Charest-Boule, Mehdi, Sandor, 1978; Feldman, 1978; Greenstein, 1978; Kraft, Hodgson, Funder, 1978; Krall, Mori, Tuck et al., 1978; Pujol, Bayard, 1979).

Physiologic effects of steroid hormones are realized by means of an induction of DNA-dependent synthesis of RNA molecules in the target cells. Glucocorticoids affect the state of nuclear and mitachondrial genomes of cytoplasmic and mitachondrial apparatus for protein synthesis in the cell (Kolpakov, 1978).

This universal mechanism of steroid action has totally confirmed the idea of biochemical processes uniformity according to which the main biochemical reactions, the cell-growth-and-division depend upon, are the same or almost the same in all the cells alive, namely, in the bacterial, plant and animal ones (Watson, 1978).

Basing on this fact we may draw an analogy to the data on the endocrine system physiology which has been studied rather well in the vertebrate animals when discussing the information on the quantitative distribution of glucocorticoids in the tissues of some invertebrates, particularly, in the short-finned squid (which is necessary for an approach to the investigation of steroid hormone function in the invertebrate animals).

Cephalopod molluscs are one of few groups of the invertebrates which have real endocrine organs (Fedotov, 1966; Fingerman, 1974; Prosser, 1978). The common octopus has been most studied as to the endocrine system. The moment of maturity is controlled by the gonadotrophic hormone of optical gland in this species (Mangold, Lanbier-Bonichon, 1975). Ovary, oviduct size increase, protein synthesis in ovary, etc., are controlled by the above-mentioned glands (Wells, Wells, 1975; Wells, O'Dor, Buckley, 1975). An extract of optical gland from the common octopus increases protein synthesis in the gonads of short-finned squid males and females (Rowe, Leslia, Idler, 1975).

The information on cephalopod molluscs steroid hormone is limited to the constatation of 17-β estradiol presence in the Loligo pealei gonad (Hagerman, Wellington, Villee, 1957) and that of endogenic testosterone in the Sepia officinalis gonad (Carean, Drosdowsky, 1973). Progesterone and testosterone have been quantitatively determined in the gonads and liver of cephalopod molluscs four species in our laboratory (Nikitina, Savchenko, Kogan, 1977). Glucocorticoids in the tissues of this class representatives has not been described, with the exception of one species, Ommastrephes pteropus, studied by our laboratory scientists (Nikitina, Taits, Kudikina, et al., 1977).

In this connection, the aim of our work has been glucocorticoid extraction and obtaining of the quantitative data on its content variations in different stages of life cycle in the organs of short-finned squid reproductive system.

Material and Methods

The material for the paper was collected in the AtlantNIRO scientific cruise undertaken to the Northwest Atlantic waters of the Nova-Scotian Shelf area in summer period, 1977.

The animals were fixed by the freezing at -18°-20°C during 30 minutes and were kept in this condition during 1-3 months. The biological analysis of the animals was carried out after their defrosting by the standard method accepted in the AtlantNIRO laboratory for commercial invertebrates (138 females and 162 males of short-finned squid). The organs of the reproductive system were extracted, weighed and homogenized to determine glucocorticoids in the animals under study. As a result, 60 samples were collected for a biochemical analysis. The homogenates obtained were extracted twice by acetone double volume: for the first time during 1-2 days, and for the second time during 3-4 hours. Then acetone was distilled off. The sediment remained was extracted twice by ethyl ether double volume. Oily sediment obtained after ether distillation was further purified by the thin layer chromatography methods on aluminium oxyde, silicagel and Silafol UV-254 plates.

As we determined besides glucocorticoids progesterone, testosterone and oestrogens, the first stage was a preliminary dispersal of all the hormones under investigation on the unfixed layer of aluminium oxyde in the chloroform: acetone (48:2) system in progesterone, testosterone, oestrogens and glucocorticoids standards presence. The standards were defined by the ultraviolet light action. The aluminium oxyde zones were taken off from the plate according to the standards. Since glucocorticoids and oestrogens were not separated enough in the above-mentioned system they were taken off as a sum.

Glucocorticoid and Oestrogen Separation

Aluminium oxyde adsorbing oestrogens and glucocorticoids was eluted by the absolute ethanol (15-30 ml). Disolvent was evapor-

ated under vacuum (T +76°C). The sediment was dissolved in chloroform and diposited quantitatively on the plates with a thin layer of silicagel. Chromatography was carried out in chloroform: :acetone:methanol (48:1:1) system in presence of oestrogens and glucocorticoids standards. Silicagel zones corresponding to the standards were taken off into separate test-tubes. An additional cleaning was done similarly on the Silufol UV-254 plates when necessary.

Glucocorticoid Determination

2.5 ml of ethanol-concentrated sulfuric acid (1:1) mixture was poured over silicagel which contained hydrocortisone and corticosterone. Then it was kept during an hour to obtain colour after which the centrifugation took place, the centrifugalized deposit being fluorometred at wave length of 471.3 nanometres. Hydrocortisone and corticosterone concentrations in the samples were determined by a calibration curve.

Results

Hydrocortisone and corticosterone have been separated from the nidamental glands and gonads of short-finned squid females in the II and III stages of maturity, as well as from the spermatophore sacs and gonads of males in the II, III, IV, V stages of maturity by the above-mentioned methods.

Maximum concentrations of hydrocortisone and corticosterone were found in the spermatophore sacs of males in the II stage of maturity (0.97 μg/g) and in the gonads of females in the II stage of maturity (0.95 μg/g). Minimum concentration of glucocorticoids was found in the spermatophore sac of a male in the V stage of maturity (0.0053 μg/g).

The hydrocortisone concentration in male gonads and spermatophore sacs has been decreased as the reproductive system has been matured. Particularly sharp decrease in glucocorticoid concentration has taken place with a transition from the II stage

of maturity to the III one. The concentration in gonads has been decreased from 0.695 μg/g to 0.049 μg/g and in spermatophore sacs from 0.97 μg/g to 0.02 μg/g. In the IV and V stages of maturity the hydrocortisone concentration has been decreased to 0.0053 μg/g. Concentration increase in the gonads has been observed with a transition from the III stage of maturity to the IV one, and then the concentration being decreased again. In the V stage of maturity only 0.017 μg/g of hydrocortisone has been contained.

We have the data on glucocorticoid concentration in the short-finned squid only for the earlier stages of maturity. An analysis of those data evidences to the fact that glucocorticoid concentration also decreases with the transition from the II stage of maturity to the III one, being especially sharp in gonads (from 0.95 μg/g to 0.26 μg/g). In the nidamental glands hydrocortisone concentration does not decrease so sharply when transiting to the III stage of maturity (from 0.39 μg/g to 0.26 μg/g) (fig.1).

Concentration decrease is generally less sharp at the earlier stages of maturity of the female reproductive system organs than that of males.

We have not got managed to trace back totally the variation of glucocorticoid concentration in the range of one stage of maturity in the animals of different size groups, although rather distinct picture has been observed for the females at the II stage of maturity (fig. 2).

Discussion

The picture described quite agrees with the phenomenon that we have observed for a number of other cephalopod molluscs, Loligo vulgaris, Todaropsis eblanae, Octopus vulgaris, for which we have obtained more comprehensive data on the glucocorticoid concentration variations, as well as on sex hormones variation during the life cycle. In particular, glucocorticoid concentration decrease in the gonads and the nidamental glands has been shown

for the allied species, Todaropsis elbanae, as the animals mature (in press). One may assume that the concentration is to be varied in the same way in the short-finned squid females.

The reproductive system stages of maturity II and III correspond to the period of oocyte protoplasmatic growth (Burukovsky, 1977; Burukovsky and Froerman, 1980, in press) when the follicular membrane is intensively formed. This period is the most long by time duration. Glucocorticoid concentrations are maximum ones. It can be explained by their influence upon the transcription process (an intensive formation of matrix RNA, ribosomal RNA, transport RNA, activization of the appropriate protein synthesis). Glucocorticoid main function in the gonads of the IV stage of maturity of the reproductive system is probably their share in providing of reserve nutrients synthesis. An intensive protein synthesis in the ovaries has been also observed for octopus, Octopus vulgaris (Wells, Wells, 1975). Spermatogenesis which takes place in the testis is mainly alike to the oogenesis by its functions (Ibert, 1969). So a variation of concentration in the process of maturing in males does not differ greatly from that of females, although it has its own characters.

In both cases glucocorticoids take part in the regulation of energy accumulation: for females in the form of nutrient reserve in the follicle, and for males to provide spermatozoid mobility. Great amount of glycogen is accumulated around the spermatozoid head at the end of spermatogenesis (Arnold, Williams-Arnold, 1978).

A suggestion of glucocorticoid functional role in oogenesis and spermatogenesis has been made basing on a general information on the role of these hormones, although we have not obtained data on their physiologic significance in the gonads of the vertebrates. This may be indicative for a more general functional role of glucocorticoids in the invertebrate animals.

An idea of interconnection between the neuroendocrinal mechanism and the process passing in the reproductive system may be

readily seen in the up to date literature on the cephalopod molluscs endocrinology. In particular, the gonadotrophic hormone of the optical gland controls the development of sexual tract, epoophoron glands and gonad maturing influencing the mitosis and regulating the meiosis. Gonadtrophic hormone is also required for the normal process of vitellogenesis (Richard, Lamaire, 1975). The optical gland extracts in the octopus ovary culture stimulate the absorption of marked amino acids and protein synthesis (Wells, 1976).

As suggestion has been made as to the fact that optical gland-subpendiculate lobetestis (gonad) system is analogous to hypothalamus - hypophys - gonad - hypothalamus system in the mammals (Wells, Wells, 1969). Glucocorticoids are not accumulated in the gonads of the vertebrates in contrast to the cephalopod molluscs, and, in particular, to the short-finned squid. The vertebrate animals have the endocrine organ, that is the adrenal cortex, to synthesize glucocorticoids. A physiologic significance of glucocorticoids is controlled by hypothalamus - hypophys - adrenal cortex system. The existence and functioning of glucocorticoids in the reproductive system organs of short-finned squid and other cephalopod molluscs allow to suppose that the neurohormone effect upon the reproductive system is produced by means of glucocorticoids, although ACTH analogues for vertebrates have not been found yet in cephalopods.

The data refering to glucocorticoids for the representatives of other mollusc classes (gastropods and bivalved) are indicative to the fact that the adaptation to the continiously varying habitat conditions takes place with glucocorticoid participation. In particular, glucocorticoid concentrations considerably increase with the unfavourable conditions. This function belongs to the adrenal cortex too in the vertebrates, corticosteroid content in blood being increased in stress situation (Baranov, 1979). Therefore, glucocorticoid level may characterize favourable or unfavourable condition of organism - environment system.

The paper is the first stage in the investigation of quantitative content and redistribution of steroid hormones in different organs and tissues of short-finned squid which is an important commercial species. At present, the work is being done which will permit to study in detail the dynamics of glucocorticoid and sex hormone content in the very different organs and tissues at different stages of life cycle. This, in turn, probably will permit to elucidate their functional significance for the cephalopod molluscs and, therefore, to relate with such important problems of rational fishery as survival, fecundity, adaptation, etc.

Conclusion

A quantitative analysis of glucocorticoids in the organs of the short-finned squid reproductive system has shown that the concentration of these hormones decreased as squids were maturing. Concentration decrease is more sharp in males than in females at the earlier stages of maturity.

In oogenesis and in spermatogenesis glucocorticoids probably take part in regulation of energy accumulation: in females in the form of reserve nutrients, and in males to provide spermatozoid mobility. One may also assume that the neurohormone effect upon the reproductive system of short-finned squid and other cephalopod molluscs is made by means of glucocorticoids.

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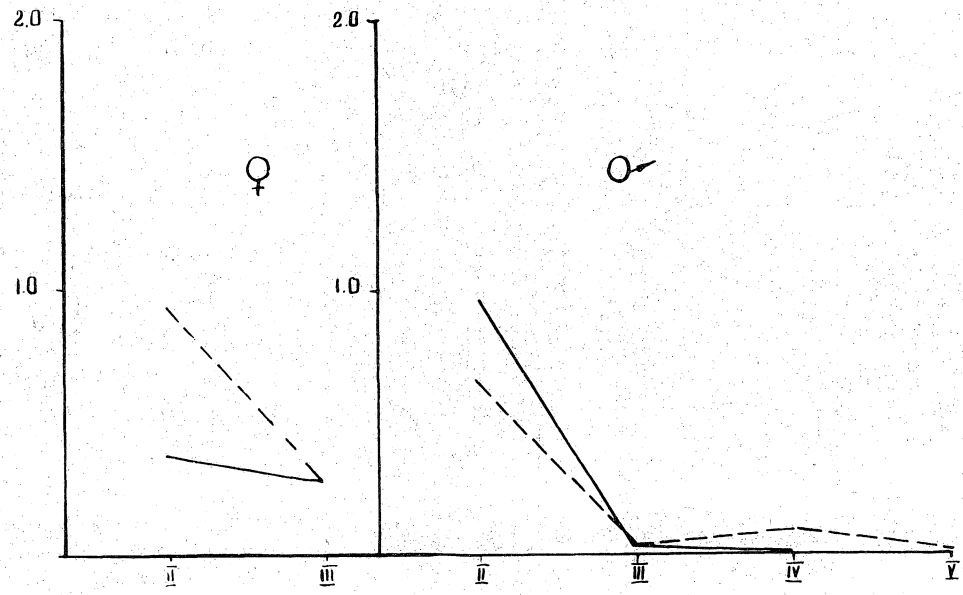

Fig. 1. Hydrocortisone concentration variations in the reproductive system organs of short-finned squids as they mature.

Symbols:

- – – – – hydrocortisone concentration in gonad
- ——— hydrocortisone concentration in nidamental gland
- ——— hydrocortisone concentration in spermatophore sac.

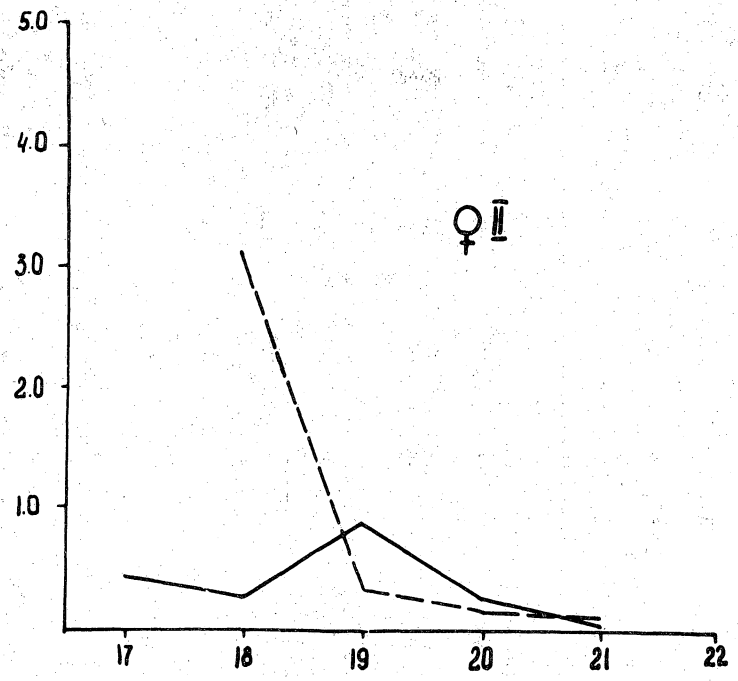

Fig. 2. Hydrocortisone concentration variations in the reproductive system organs of short-finned squid females as they mature.

– – – – – hydrocortisone concentration in gonad

——————— hydrocortisone concentration in nidamental gland.