NOTE

Modulation of glial fibrillary acidic protein immunoreactivity during *Sparus aurata* L. development. A preliminary report*

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SUMMARY: A band of approximately 50-51 kDa, corresponding to glial fibrillary acidic protein (GFAP), was detected by immunoblot techniques in the brain tissue of *Sparus aurata* L. The levels of GFAP immunoreactivity were determined at three different stages of development: 6 month old, 18 month old and adults reared in an aquaculture facility for 3-4 years. Our results indicated a decrease of GFAP levels during *S. aurata* development from six month old to adult individuals. An explanation for this observation is suggested.

Key words: *Sparus aurata*; Glial fibrillary acidic protein; GFAP; immunoblot; Western blot; Marine fish development.

RESUMEN: MODULACIÓN DE LA PROTEÍNA ACÍDICA FIBRILAR GLIAL DURANTE EL DESARROLLO DE *SPARUS AURATA* L. — Una banda de aproximadamente 50-51 kDa correspondiente a la proteína ácida glial fibrilar (GFAP) fue detectada mediante técnicas de inmunoblot en tejido cerebral de *Sparus aurata* L. desarrollado en piscifactoría. Se determinaron los niveles de GFAP en tres diferentes estados de desarrollo: especímenes de 6 y 18 meses de edad y adultos mantenidos en cautividad durante 3-4 años. Nuestros resultados indican una disminución de los niveles de GFAP durante el desarrollo de *S. aurata* de 6 meses a adulto. Se sugiere una explicación para esta observación.

Palabras clave: *Sparus aurata*; proteína ácida fibrilar glial; GFAP; immunoblot; Western blot; desarrollo de pez marino.

INTRODUCTION

The gilthead seabream, *Sparus aurata* L. (Pisces: Sparidae), is an important component of the littoral ecosystems throughout the Mediterranean. It is abundant in waters shallower than 60 m and in brackish water lagoons. *S. aurata* is found from England to Senegal, but it is not commonly observed in Atlantic coastal waters. Due to its high commercial value it is currently reared in industrial aquaculture facilities. Aquaculture facilities also provide an important tool for aquatic biologists to study developmental and ecological parameters of fish development.

The glial fibrillary acidic protein (GFAP) is the main constituent of intermediate filaments of mammal astrocytes (Eng et al., 1971; Eng, 1985). Immunological and charge properties of this protein have remained quite constant from fish to mammals (Mencarelli et al., 1993). Few studies have been done on the glial component of the fish central nervous system (CNS) due in part to the difficulty in
identifying fish astrocytes by immunohistochemistry (Dahl et al., 1985; Maggs and Scholes, 1986). Recently the use of monoclonal antibodies to porcine GFAP has become a tool to detect GFAP positive astrocytes in fish CNS (Blaugrund et al., 1991). Modulation of GFAP levels have been detected in fish neural tissues in different systems. Injury to the carp (Cyprinus carpio) optic nerve was followed by a decrease of GFAP immunoreactivity (Blaugrund et al., 1991) and a transient decrease of GFAP immunoreactivity was detected in several brain areas of the goldfish (Carassius auratus) rhombencephalon after experimentally induced hyperammonemia (Rubio et al., 1993). In this paper we examined the developmental profiles of GFAP immunoreactivity in brain tissue of aquacultured S. aurata in an attempt to provide basic data on CNS and ageing processes in marine fish.

MATERIAL AND METHODS

Larvae were obtained from naturally spawning adult S. aurata kept at the “Es Murterar” Aquaculture Station (Mallorca, Balearic Islands) under photoperiod conditions appropriate to stimulate spawning in December. Fertilised eggs were placed in incubation boxes, and larvae hatched after 40 h at 19-20°C. All the larvae hatched over a period of 2 h. Larvae were reared in a 18 m³ tank with recirculated seawater at a concentration of 40 larvae/l. Water temperature was maintained at 20°C and photoperiod was regulated at 14 light hours: 10 dark hours (14L:10D).

The feeding was adapted to optimal larval development at 20°C (Quillet and Camaret, 1982). The rotifer, Brachionus plicatilis, was provided at a density of 10-20 rotifers/ml from day three. Nauplii of Artemia spp. were provided from day 14 at a density of 8-10 Artemia f/ml. The chlorophycean algae, Tetraselmis sp., was added from day three to ensure water quality. After day 30 the larvae were fed Artemia metanauplii and artificial food. After day 60 the juveniles were fed artificial food and situated in outside mesocosms (volume = 110,000 litres) with natural photoperiod and regulated water temperature. The artificial diet Lancy Artemia Systems was provided by means of demand dispensers. In June 1994, specimens born in December 1993 (6 month old) and specimens born in December 1992 (18 month old) were sampled and immediately placed in a mixture of water and ice at 0°C. Each age group proceeded from a single female spawning. Three adult fishes which have been in the Aquaculture facility for 3-4 years were also sampled. The mean length of 6 month old fishes was 22.66 ± 1.16 cm corresponding to a mean weight of 229.33 ± 40.16 g. While for 18 month old fishes the mean length was 26.35 ± 1.41 cm and the weight 381.20 ± 48.02 g. The three adult fishes ranged from 37 to 50.5 cm corresponding to 871, 983 and 3040 g. The macrostructural morphological observation of the gonads resulted in all the juvenile fishes having male morphology while adults were females.

Immunoblots were performed as described elsewhere (Beitner-Johnson et al., 1993) with modifications. Briefly, 100 to 200 mg of whole brain was homogenised (1:20 w/v) in 40 mM Tris buffer, pH 6.7, containing 1% Triton X-100, 1 mM EDTA, 1 mM phenylmethylsulfonylfluoride and leupeptin (40 μg/ml). The samples were centrifuged at 40,000 g for 45 min at 4°C and the supernatant was subjected to electrophoresis (SDS-polyacrylamide gel electrophoresis) in a 10% polyacrylamide gel. Proteins were determined by the method of Bradford (1976). Separated proteins were transferred to nitrocellulose membranes blocked in a phosphate-buffered saline containing 5% non-fat dry milk and 0.1% Tween-20 (blocking solution) and incubated at room temperature for 2 h in blocking solution containing the primary antibody (anti-GFAP monoclonal antibody, Sigma; clone GA5) at a 1:5000 dilution. The secondary antibody, (horseradish peroxidase-linked sheep anti-mouse IgG; Amersham International; Buckinghamshire, U.K.) was used at 1:5000 dilution. Immunoreactivity was detected with an enhanced chemiluminescence (ECL) western blot detection system (Amersham international) followed by exposure to Amersham Hyperfilm ECL. Films were scanned in the image analyser Bio Image (Millipore, Ann Arbor, MI). The IOD corrected by the protein content (in the range of linearity) is termed IOD unit.

Fish length, weight and experimental results are expressed as mean ± S.E.M. values.

RESULTS

Some adult fishes of reproductive age were obtained by long-line fishing and maintained in the Aquaculture plant with a diet composed of natural crushed food (Mittillus sp. and fish) and artificial food.

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A main immunoreactive band was detected in *S. aurata* brain tissue after immunoblotting with an anti-porcine GFAP monoclonal antibody (Fig. 1). The relative molecular mass (50-51 kDa) of this protein signal is in the range of the immunoreactive band present in rat brain (Fig. 1), previously described as the monomeric form of this cytoskeletal protein (Eng, 1985). This result indicates the specificity of the GFAP immunoreactivity signal in brain tissue of *S. aurata*.

**DISCUSSION**

One way to study glial cells is through investigation of the intermediate filaments (GFAP) they express, since they are indicative of the differentiation and maturation stage of the cell. In our experiments, immunoblot analysis of the brain tissue of *S. aurata* with anti-porcine GFAP monoclonal antibody (Sigma) detected a main protein signal (band) of a relative molecular mass of approximately 50-51 kDa. The molecular mass of this band is in the range of the immunoblot protein signal detected in rat frontal cortex and previously described as corresponding to GFAP (Eng *et al.*, 1971; Eng, 1985). Similar values of molecular mass (52 kDa) have been described in goldfish (Carassius auratus) neural tissue (Blaugrund *et al.*, 1991).

This study shows that GFAP levels decreased during the development of *S. aurata*. However, since this study has been performed using a single female spawning to form each age group, genetic factors could account in part for the present results. The interpretation of our results is also limited because an incomplete understanding of the role of GFAP in brain function. There is growing evidence that glia cells play an important role in the physiology of the...
developing and adult brain. In the developing brain neurons migrate to their specific targets using radial glia projections, preserve the ionic composition of the extracellular space and maintain the blood-brain barrier (Arenander and De Vellis, 1989; Kimelberg and Norenberg, 1989; Raine, 1989). In addition, astrocytes can release various growth factors for neurons (Englele and Bohn, 1991) and also provide an aid in synaptic transmission (Martin, 1992). In mammal brain development, neurogenesis is virtually complete during the fetal and early postnatal periods. Moreover, the ageing process in mammals is associated with a loss of neuronal mass and an astrocyte hypertrophy concomitant with a marked increase of GFAP mRNA levels and protein (Goss et al., 1991; O’Callaghan and Miller, 1991).

In fish CNS (and other phylogenetically lower vertebrates), new neurons are continuously being added throughout life. Nevertheless the rate of neurogenesis seems to decline as the fish ages. This effect was seen in goldfish retina (Easter et al., 1981). Recently it has been demonstrated that astrocytes play an important role in the control of neuronal proliferation in brain cell cultures of goldfish (Carassius auratus) by (i) direct astrocyte-neuron contact and (ii) by secreting soluble factor(s) that inhibit neurite extension providing an additional signal for proliferation of neurons (Sivoron et al., 1993). In addition differentiating neurons secrete soluble factor(s) that inhibit astrocyte proliferation. These results are consistent with our observation of a loss of GFAP immunoreactivity, thereby loss of astrocytes, astrocytic projections or filaments, during S. aurata development. It is conceivable that at an early developmental stage of S. aurata (6 months after hatching when there are high values of GFAP immunoreactivity) there is the need for a relatively abundant mass of astrocytes to sustain the high rate of neurogenesis occurring at this stage of fish development. An increased GFAP immunoreactivity is therefore expected.

Specimens of 3-4 years old, low levels of astrocytic mass (GFAP) were detected in our experiments (because of the low number of specimens and sex, adults were exclusively females whereas juveniles were males, we did not include this group in the statistical analysis). This result is consistent with the fact that adult fishes present relatively lower rates of neuronal proliferation. According to this rationale, developing specimens (18 month old) should present intermediate levels of GFAP. In our experiments, the GFAP levels at this developmental stage were distributed into two groups as seen in the pattern of the immunoreactivity values. One possible explanation of this peculiar distribution of data in the 18-month group is provided by recent evidence suggesting that astrocytes may be involved in mechanisms of synaptic plasticity driven by gonadal hormones. Modulation of GFAP immunoreactivity has been recently detected in brain tissue during the strous cycle in female rats (Garcia-Segura et al., 1994).

S. aurata are protandric hermaphrodites. They mature at the end of the first year of age as males. Fishes of 17 months old undergo a sexual change and, the majority (~80%) will become females. The rest (~20%) remain as males but many change sex as they grow older. The old specimens are predominantly females (Quillet and Camaret, 1982). By 18 month of age, morphological differences between both sexes are not evident but the sex-specific gonadal hormonal changes may be underway and modulate glial plasticity, number of astrocytes or astrocytic projections in the brain tissue. This fact could result in sex-specific levels of GFAP immunoreactivity at this stage of fish development. Our results are the first determination of CNS parameters in a marine fish and have shown a relationship between age and GFAP modulation. Moreover, the possible relationship between sexual changes and GFAP levels provide a new tool to study the regulatory effects of sexual hormones upon CNS in fish. The relationship between GFAP levels in brain tissues and sexual development in S. aurata will be completely studied.

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REFERENCES


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