Role of the pineal organ and melatonin in fish

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The pineal organ is a part of the central nervous system that is formed - like a retina - as an evagination from the embryonic features. Although it was once inferred that the pineal organ of teleost fish was a sensory organ (Studnicka 1905), the first direct evidence for its photosensitivity was obtained from studies conducted on rainbow trout by Dodt (1963). During the following decade, extensive comparative electron microscopical and neurophysiological studies laid the foundation for our current understanding, that during vertebrate evolution the pineal organ has transformed from a photosensory organ into an endocrine gland (Collin 1969).

In mammals it has been shown that the pineal organ (Figure 1) synthesizes the indoleamine melatonin and, not much later, it was demonstrated that melatonin synthesis was indirectly controlled by the daily dark-light cycle (Axelrod 1974). Moreover, it was demonstrated that melatonin is a major mediator in the photoperiodic control of annual reproductive cycles (Reiter 1991).

After the first demonstration of an enzyme involved in melatonin synthesis by the pineal gland of rainbow trout (Quay 1965), much research has been devoted to the physiological role of the pineal organ and melatonin in fishes. It is clear that the pineal organ of fish is a photosensory organ that synthesizes the “photoperiod-mediating” hormone melatonin, that it may convey information by means of neural and hormonal signals and that it contains a circadian oscillator (Figure 1).

Pineal Anatomy

In most poikilothermic vertebrates, the pineal complex has two components. In fish, they are the pineal and parapineal organs (Ariens Kappers 1965). The pineal organ is often differentiated into a proximal slender pineal stalk and a distal expanded end vesicle. In some species, the adult pineal organ is very large and covers the entire telencephalon. It is currently agreed that the photoreceptor cells are the source of the melatonin produced by the pineal organ. The cerebrospinal fluid (CSF) of the ventricular system is, thus, potentially a means of transport for various chemical compounds between the pineal organ and the brain. Substances synthesized in large amounts by the pineal organ, such as melatonin, may reach the brain by diffusion through the CSF.

Cellular Components of the Pineal Organ

The distinguishing features are found in the apical (receptor) and the basal (effector) poles. The apical pole contains the outer segment, which contains the light sensitive photopigment, and the inner segment, containing numerous mitochondria and longitudinal cytoskeletal elements. The basal pole is the photoreceptor axon, which forms contacts with the basal lamina that surrounds the pineal parenchyma. The apical pole may vary in shape and size. The outer segment lamellae may form a regular cone-shaped stack similar to that of retinal cone photoreceptors. The inner segment is typically a short, rounded structure packed with mitochondria. Melatonin is a highly diffusible lipophilic molecule that cannot be fixed by conventional chemical fixatives.

Interstitial cells

A large proportion of the cells of the pineal parenchyma consist of supportive or interstitial cells. They are located between the photoreceptor cells, to which they are attached by tight junctions that form a diffusion barrier between the CSF of the pineal lumen and the extracellular fluid surrounding the photoreceptor cell bodies, axons and intrapineal neurons.

Macrophages

Macrophages are present in the pineal lumen in several species, often in close contact with photoreceptor outer segments. Specifically, macrophages seem to express a circadian rhythm in the activity of lysosomal enzyme acid phosphatase, which may be involved in the breakdown of phagocytized and shed outer segment membranes.

Melatonin Synthesis

The synthesis of melatonin by the pineal organ is regulated by the intensity of the ambient illumination and reaches the
The effect of pineal removal on circulating melatonin levels in Atlantic salmon parr

The production of melatonin by the pineal exhibits a distinct diurnal rhythm with elevated levels during the hours of darkness (Randall et al. 1995). Inasmuch as both smoltification and maturation in Atlantic salmon, Salmo salar, are known to be under the influence of photoperiod, a simple and effective method of pineal removal was developed for use in reducing nighttime melatonin levels in young salmon in future long-term experiments.

In this experiment, 30 one-year-old salmon parr were maintained under a constant LD 12:12 photoperiod at ambient temperature. The fish weighed an average of 55.3 g at the time of surgery. The were anesthetized in a solution of 2-phenoxyethanol (0.05 percent v/v). A 5 mm horizontal incision was made posterior to the pineal window, which is situated within the cranial bone and clearly visible in young salmonids through the skin. Then, a flap of tissue was lifted anteriorly to reveal the pineal gland. The pineal stalk was cut at the point of attachment to the diencephalon, the gland removed with forceps and the area cleaned by suction, using a pipette. The overlying tissue was replaced and a 3:1 mixture of orahesive powder and cicatrin antibiotic applied over the incision. A seven percent mortality rate was observed in pinealectomized fish and fewer than one percent mortalities in the control. After an interval of three months, external examination of the two groups did not reveal any observable differences. The nocturnal increase in circulating melatonin in the Atlantic salmon, derived principally from the pineal and the pinealectomy procedure, is an effective method of reducing nighttime melatonin levels in young salmon.

Role of Pineal Organ in Reproduction

Numerous attempts have been made to analyze the role of the pineal organ in the photoperiodic control of reproduction. The experimental approach has usually been to perform extensive studies of the effects of pinealectomy and/or melatonin administration under different photothermal regimes at specific stages of the reproductive cycle. Different species show varied sensitivities to pinealectomy and/or melatonin administration at similar reproductive stages or photothermal regimes.

The effects of pinealectomy vary with species and with season within species. Pinealectomy apparently affects the timing of reproduction, as demonstrated by effects on gonadal maturation and post-spawning phase. The circulating sex steroid levels showed the effects of pinealectomy on the gonads.

The seasonal reproduction of rainbow trout and other salmonids ensures optimal survival of the offspring. Changing photoperiod is a major factor in the timing of reproductive activity in salmonids (Henderson 1963, Handersen et al. 1992). In seasonally breeding mammals, the pineal hormone, melatonin, is synthesized and secreted nocturnally, in a manner that directly reflects the length of the dark phase. The melatonin signal, therefore, provided a hormonal template of ambient photoperiod and influences both daily and seasonal patterns of behavior, including reproduction (Reiter 1993).

A general observation was that daily melatonin injections attenuate ovarian development induced by long daylighted conditions (Joy and Khan 1991). In female catfish, Heteropneustes fossilis, melatonin exerted an antigonadotropic effect during the late preparatory prespawning and spawning periods (Sundararaj and Keshavanath 1976) that may have been mediated by the hypothalamic serotonergic system (Senthil-kumaran and Joy 1995). In contrast, according to Garg (1989), melatonin did not affect ovarian activity or vitellogenin levels during the preparatory and prespawning periods, whereas pinealectomy did so during the preparatory and postspawning periods only. In male H. fossilis, melatonin administration did not affect
the histological appearance of pituitary gonadotrophs, testes or seminiferous tubules in the prespawning season (Keshavanath 1981), possibly indicating a gender-dependant difference in the response to melatonin.

In Atlantic salmon and rainbow trout, administration of constant release melatonin implants did not affect timing of spawning, although pinealectomy did affect timing, indicating the importance of cyclicity with low daytime melatonin levels.

Regarding the nature of the signals from the pineal organ, melatonin often attenuates progonadal effects of long days. However, there is currently little evidence to support a major role for melatonin in the control of reproduction in teleosts.

**Growth and Metabolism**

Photoperiod and temperature control seasonal changes in lipid metabolism and growth. The pineal organ appears to be involved in the mediation of photoperiod and temperature signals. In goldfish, pinealectomy induces a reduction in body weight and linear growth.

The effect of daily administration of melatonin for 20 days was evaluated with respect to the response of hypophysial-ovarian system in *H. fossilis* (Sundararaj and Keshavanath 1976). In the prespawning period, daily treatment with melatonin (20-100 μg/fish) significantly inhibited vitellogenesis and induced follicular atresia in the ovary. During the spawning period, melatonin (20-100 μg/fish) treatment induced significant ovarian regression.

A comparison of ovarian recrudescence in the catfish, *Mystus tengara* (Ham) exposed to melatonin was also observed by Saxena and Anand (1977). The treatment with melatonin was more effective in arresting ovarian recrudescence.

Several efforts have been made to discern whether the pineal organ is involved in regulation of seasonal changes in carbohydrate metabolism (Delahunty *et al.* 1978, 1980, Delahunty and Tomilson 1984a,b). John *et al.* (1980, 1983) investigated the effects of intraperitoneal injections of melatonin on plasma glucose and free fatty acids levels in adult rainbow trout.

**Effect of melatonin implants on the levels of testosterone and development of ovaries in tilapia**

The pineal gland has a role in the stimulation of gonadal activity in fishes. The production of melatonin by the pineal is high during the hours of darkness and the level of melatonin secretion is low during daylight. It was demonstrated that melatonin synthesis is indirectly controlled by the dark-light cycle. Excess secretion of melatonin restricts the development of the gonad.

In this study, the effects of melatonin implants on gonadal activity of *Oreochromis aureus* was investigated. On average, 300-400 g fish were selected for the experiment. The selected fish were tagged with electronic tags before conducting the experiment. The experiment was conducted for 60 days. Before implantation, an incision was made in the dorsal side of the fish to keep the hormone pellets intact. Twenty fish were implanted with Regulin melatonin implants and twenty fish were kept as controls. Blood samples were taken every two weeks for testosterone analysis.

In the first sample, the average testosterone level was 2.04 ng/ml plasma in control and 2.64 ng/ml plasma in implanted fish. In the second sample, the average testosterone level was very low in implanted fish, but the level was high (18.55 ng/ml plasma) in control fish. The testosterone level was lower again in the third sample (2.66 ng/ml plasma) of the implanted fish. In the control, it was 17.43 ng/ml plasma. Apart from testosterone analyses, ovary samples were taken every two weeks for histological examination. Development of ova was reduced in melatonin-implanted fish. This shows that it is possible to control maturity through melatonin implants. Melatonin secreted by the pineal organ has the capacity to restrict the secretion of gonadotropin from the pituitary gland. A low level of gonadotropin controls development of maturity. Implantation of melatonin pellets causes increased blood melatonin levels. A melatonin surge in the blood indirectly controls the development of the ovaries.

**References**


Fenwick, J.C. 1970. The pineal organ: (Continued on page 69)
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