POINTS OF VIEW

Arginine vasotocin–melatonin interactions in fish: a hypothesis

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Arginine vasotocin and melatonin in fish – short presentation

Arginine vasotocin (AVT) and isotocin (ichthyotocin) are two nonapeptides produced, according to osmotic stimuli, in separate hypothalamic neurosecretory neurons in teleost fish. The neurosecretory cells concentrated in the preoptic nucleus (NPO) give rise to separate vasotocinergic and isotocinergic axons, which end in the neurohypophysis, where the hormones are stored and released (Goossens *et al.*, 1977; Van den Dungen *et al.*, 1982).

In fish, AVT plays a role in osmoregulation, cardiovascular activity, endocrine secretion and reproductive processes (Bentley, 1971; Henderson and Wales, 1974; Babiker and Rankin, 1978, 1979, 1980; Fryer and Leung, 1982; Pang *et al.*, 1983; Rodriquez and Specker, 1991). Furthermore, the widespread distribution of AVT-immunoreactive fibres and AVT binding sites in various regions of the brain suggests that AVT may act as a neurotransmitter and/or neuromodulator in the central nervous system in fish (Goossens *et al.*, 1977; Van den Dungen *et al.*, 1982).

Arginine vasotocin and isotocin are closely related with the mammalian vasopressin (AVP), and vasotocin is regarded as the original parent neurohypophysial hormone in vertebrates (Fig. 1, upper).
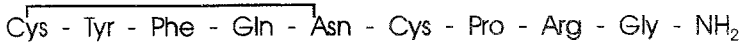
Several lines of evidence indicate that AVT and AVP are synthesized *de novo* also in the mammalian pineal (Binkley, 1988; Olcese *et al.*, 1993) and AVT has been detected in the fish pineal (Holder *et al.*, 1979; Binkley, 1988).

Melatonin is a product of tryptophan metabolism by the pineal gland and retina in vertebrates. In all species examined, melatonin synthesis is photoperiod dependent and shows a diurnal rhythm with high levels during the night-time and low levels during daytime (Reiter, 1991; Skene *et al.*, 1991). In teleosts, the pineal containing photo-receptor cells is considered to be the major source of melatonin synthesis (Kezuka *et al.*, 1992; Zachmann *et al.*, 1992b) and is involved in the control of rhythmic adaptations to daily and seasonal cycles (Morton and Forbes, 1988; Falcon and Collin, 1989; Underwood, 1989; Iigo *et al.*, 1991; Zachmann *et al.*, 1991, 1992a,b; Max and Menaker, 1992).
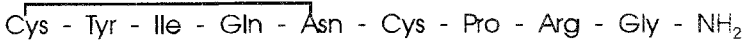
Why AVT–melatonin interactions in fish are considered

Many species of teleosts exhibit seasonal reproductive and feeding migrations between waters of markedly different salinities. In some fish species, photoperiod and temperature
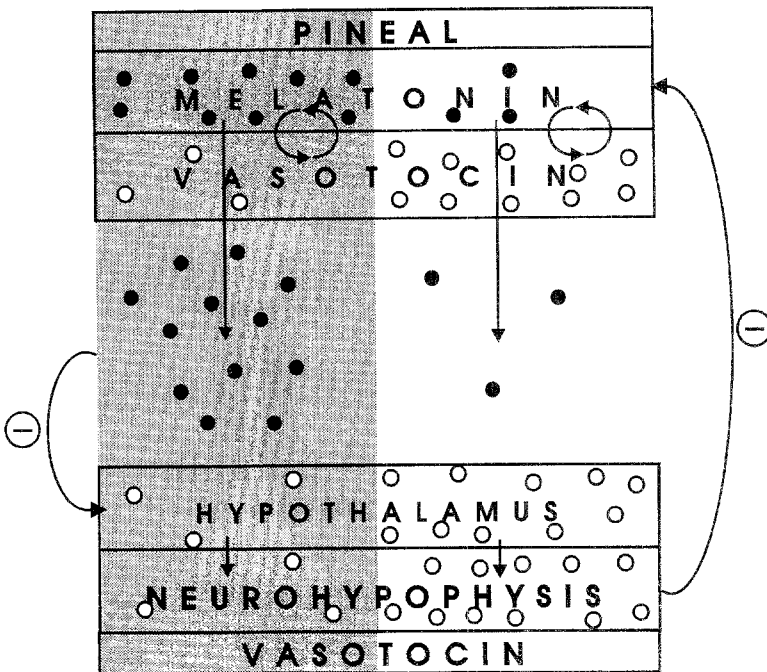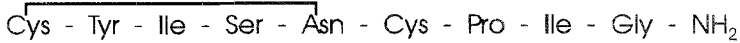
ARGININE VASOPRESSIN

Cys - Tyr - Phe - Gln - Asn - Cys - Pro - Arg - Gly - NH$_2$

ARGININE VASOTOCIN

Cys - Tyr - Ile - Gln - Asn - Cys - Pro - Arg - Gly - NH$_2$

ISOTOCIN

Cys - Tyr - Ile - Ser - Asn - Cys - Pro - Ile - Gly - NH$_2$

Fig. 1. (upper) Amino acid sequence of the selected natural neurohypophysial hormones. (lower) Proposed model for AVT–melatonin relationships in fish: melatonin (●) synthesized in the pineal during darkness (shaded area) inhibits vasotocin synthesis in hypothalamic neurons and/or in the pineal; vasotocin (○) synthesized according to osmotic stimuli in hypothalmic neurons (released in neurohypophysis) and/or in the pineal inhibits melatonin synthesis in the pineal.

play a role in the state of the reproductive system and the pineal gland has been implicated in these responses to the environment (Morton and Forbes, 1988; Duston and Saunders, 1990; Zachmann *et al.*, 1991; Max and Menaker, 1992a).

The pineal is regarded as a 'photoneuroendocrine and thermoendocrine transducer'

converting photic and temperature information into hormonal signals and/or into a neural message, which is transmitted by centrally projecting intrapineal neurons to various brain areas (Ekström and Meissl, 1989; Falcon and Collin, 1989; Meissl *et al.*, 1990). The pineal synchronizes the reproductive cycle with cycles in the environment (Norris, 1980). Probably, the target organs distinguish the season from the pattern of melatonin synthesis: a short period of high melatonin synthesis in summer, and a prolonged period with increasing night length and high melatonin synthesis in autumn and winter (Binkley, 1988; Bartness and Goldman, 1989; Vanecek, 1991).

In salmonids the major environmental initiator for smolt transformation and seaward migration appears to be lengthening of the photoperiod in the spring (Norris, 1980; Duston and Saunders, 1990). Thus photoperiod affecting melatonin synthesis in the pineal may stimulate some physiological and behavioural changes in fish and may be a signal to begin migration (Norris, 1980; Boeuf, 1992).

On the other hand, migrations demand extensive osmoregulatory adjustments dependent on hormones responsible for water and electrolyte regulation (Bentley, 1971; Norris, 1980; Boeuf, 1992). One of them is AVT.

It is known that following transfer of euryhaline fish – rainbow trout (*Oncorhynchus mykiss*, Salmonidae), flounder (*Platichthys flesus*, Pleuronectidae) and medaka (*Oryzias latipes*, Oryzitidae) – from sea water to fresh water there is a rapid increase in the store of neurohypophysial AVT and in plasma AVT level, which would reflect an increase in the rate of both synthesis and release. On transfer of fish in the opposite direction a rapid reduction in pituitary stores and plasma AVT level has been observed (Haruta *et al.*, 1991; Perrot *et al.*, 1991. In rainbow trout the amount of pro-vasotocin mRNA is decreased in sea water and returns to initial levels after transfer of fish back to fresh water (Hyodo and Urano, 1991).

The mediation of environmental changes through the endocrine system to coordinate physiology and behaviour in fish implicates some hormonal interactions.

Is there a functional relationship between the hypothalamo–neurohypophysial and pineal systems in teleosts?

There are a few reasons to say probably, yes:

- as mentioned above, participation of both AVT and melatonin in a system controlling the physiological adaptation of fish to daily and seasonal environmental changes (light, temperature, salinity), especially important for migrating fish (Bentley, 1971; Norris, 1980; Binkley, 1988);
- presence of AVT in the fish pineal (Holder *et al.*, 1979; Binkley, 1988);
- presence of melatonin receptors in the fish preoptic area, the part of the brain including the nucleus preopticus, which is the site of vasotocin synthesis (Martinoli *et al.*, 1991; Ekström and Vanecek, 1992);
- analogy with AVT/AVP–melatonin interactions in mammals.

So far, AVT–melatonin interactions in fish have not been investigated. Therefore the available data from mammals are presented here and some analogies are drawn.

It is assumed that in the mammalian pineal there are two biologically active nonapeptides, AVP and AVT (Ebels and Balemans, 1986; Binkley, 1988). In the rat pineal, a diurnal rhythm in vasotocin content with a peak during daytime and a midnight

nadir, has been reported by Calb *et al.* (1977). Moreover, the laboratory rat exhibits a seasonal variation in AVT (Prechel *et al.*, 1983). It is established that reduced AVT (disulfide bond opened during the initial and rate-limiting step in the AVT conversion cascade) protects against inactivation of the pineal N-acetyltransferase (NAT), the rate-limiting enzyme in the melatonin-synthesizing pathway. On the contrary, inactivation of NAT is accelerated by disulfide molecules, e.g. AVT itself (Binkley, 1988). In addition, when rat pineals were given norepinephrine to stimulate melatonin synthesis, AVT decreased melatonin synthesis (Binkley, 1988). Accordingly, the enhancement of AVT levels may lead to a suppression of melatonin synthesis. This finding is also in good agreement with the correlation of a high AVT content in the mammalian pineal with a decreased melatonin synthesis during the day (Calb *et al.*, 1977). Similarly, norepine-phrine-induced stimulation of melatonin release in the bovine pineal gland is inhibited significantly by the presence of AVP in a concentration-dependent manner (Olcese *et al.*, 1993). These data suggest the participation of AVT and AVP in regulation mechanisms of melatonin synthesis in mammals.

In fish, however, it remains unknown whether there are daily and seasonal changes in AVT levels, and the physiological role of this hormone in the pineal is unclear.

The data from mammals suggest that nonapeptides may contribute to the circadian system of vertebrates. It is established that AVP neurons are located in the suprachiasmatic nucleus (SCN) in mammals (De Kloet *et al.*, 1990; Yamase *et al.*, 1991). The SCN is considered to be the 'biological clock' of the brain and it is known to control numerous cerebral and somatic circadian rhythms, including the synthesis and secretion of melatonin by the pineal gland (Morgan and Williams, 1989; Reiter, 1991). Changes in activity in the AVP neurons of the suprachiasmatic, paraventricular and supraoptic nuclei, with the minimum in darkness, result in the circadian changes in AVP levels in the cerebrospinal fluid (CSF), where the AVP is secreted (Yamase *et al.*, 1991; Windle *et al.*, 1992). The vasopressin in the CSF may have a role as the circadian signal to various brain areas. The AVP in the SCN may act as a neurotransmitter or neuromodulator in processes of signal transmission, or may be involved in the generation of the circadian rhythm (Yamase *et al.*, 1991). It is of interest to note that daily variations in vasopressin mRNA levels in the SCN in rats are also observed. This rhythmicity is unique to the SCN and does not occur in other hypothalamic nuclei. Peak levels are present at the end of the light phase, while vasopressin mRNA levels are lowest at night (Uhl and Reppert, 1986; Reppert and Uhl, 1987; Burbach *et al.*, 1988).

It is worth mentioning that in mammals, melatonin has also been shown directly to inhibit the protein synthetic activity of the SCN (Morgan and Williams, 1989). Therefore a high melatonin level during night-time corresponds with low synthetic activity in AVP neurons in the SCN, and a low melatonin level during daytime with high AVP synthesis.

It is generally presumed that in teleosts the preoptic area is the seat of the clock homologous with the mammalian SCN, although as yet there is no experimental evidence for or against this. It is highly notable that the neurons in the preoptic area are immunoreactive for AVT and isotocin (Goossens *et al.*, 1977; Van den Dungen *et al.*, 1982). Furthermore, high levels of melatonin binding have been demonstrated in the preoptic area (Martinoli *et al.*, 1991; Ekström and Vanecek, 1992).

These observations, taken together with the recent report of the effect of hypo- and hypertonic media on melatonin output and its rhythm in cultured chick pineal cells (Zatz and Wang, 1991a,b), open up the additional possibility of regulation mechanisms of

melatonin synthesis and release. It has been shown that the hypertonic and hypotonic media mimic the effect of, respectively, light and dark pulses on the chicken pineal cells. The fact that the synthesis and secretion of AVT is very sensitive to osmotic stimuli is another reason to consider the AVT-melatonin interaction.

AVT–melatonin in fish – a model

The proposed AVT-melatonin relationships in fish are presented in Fig. 1 (lower). Melatonin, synthesized in the pineal during darkness, may inhibit vasotocin synthesis in hypothalamic neurons and/or in the pineal. The low level of melatonin during light-time is insufficient to inhibit vasotocin synthesis in hypothalamic neurons and/or in the pineal and therefore the activity of AVT-neurons may be high. On the other hand, AVT synthesized according to osmotic stimuli in the hypothalamic neurons (and released in the neurohypophysis) and/or in the pineal may inhibit the synthesis of melatonin in the fish pineal. Because melatonin secretion is directly controlled by light, melatonin may regulate pineal AVT levels, with negative feedback from AVT. The rhythmic secretion of melatonin may be crucial to maintaining a rhythmic activity in the 'SCN' region of fish, in the absence of a discrete circadian oscillator.

AVT-melatonin interactions may be of great importance, especially for migrating fish, in which mechanisms of adaptation to both light and salinity play a key role. It is interesting that the migratory form of salmonid fishes (the smolt) still living in fresh water is perfectly preadapted to sea water and is able to migrate without transitory osmotic disequilibrium (Norris, 1980; Boeuf, 1992). It is possible that the smolt pineal, converting photic information into a hormonal signal, changes directly or indirectly the synthetic activity of AVT neurons in the hypothalamus and/or in the pineal and thus adjusts the osmoregulatory mechanisms in fish to salinity changes.

Acknowledgements

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