In this perspective article, we will mainly discuss results obtained in fish; however, we will occasionally cite studies on mammalian species in order to compare the differences or to corroborate our hypothesis. Most of the studies on serotonin metabolism in fish have been conducted by the teams of Nilsson and Ekström: the former group focused on the role of serotonin in stressful conditions (Winberg et al. 1993), whereas the latter group used immunocytochemical methods to examine serotonergic neurones in the brain of teleost fish (Ekström, 1987; Ekström et al. 1986; Ekström and Veen, 1984; Vecino and Ekstrom, 1990; Holmqvist and Ekström, 1995). Few studies have been conducted on the distribution of serotonin receptor subtypes in fish brain. By using autoradiographic techniques, Palacios and Dietl (1988) demonstrated the presence of 5-HT1A receptor sites in fish brain. Hansely and Cohen (1992) demonstrated the pharmacological presence of the 5-HT1A receptor subtype in goldfish retina. We have recently observed the presence of 5-HT1A receptors in rainbow trout using (±)-8-hydroxy-2-(di-n-propylamino)-tetralin (8OH-DPAT), an agonist of the 5-HT1A receptor subtype (Khan et al. 1996c). By using a similar strategy of displacing [3H]8OH-DPAT, Winberg and Nilsson (1996) have recently demonstrated the presence of at least three different high-affinity serotonin binding sites in the whole brain of the Arctic char Salvelinus alpinus. These investigators have identified one of the three serotonin binding sites as being of the 5-HT1A receptor subtype whose pharmacological profile was markedly similar to that of the mammalian counterpart. However, there is no direct evidence for the presence of the 5-HT1A receptor subtype from experiments using [3H]8OH-DPAT in fish species. Furthermore, as in mammals, the density of serotonergic receptors is higher in the telencephalon than in the diencephalon of fish brain (Khan et al. 1996c).

Much work still has to be done to characterize biochemically and pharmacologically the serotonin receptor subtypes in fish brain. The serotonergic receptors, mainly 5-HT1 (1A, 1B, 1C, 1D), 5-HT2 (2A, 2B), 5-HT4, 5-HT6 and 5-HT7, belong to a G-protein-coupled receptor superfamily and possess the seven transmembrane segments typical of such proteins (Peroutka, 1993). The 5-HT1A receptor subtype is coupled either with adenylate cyclase or with phospholipase C, depending on cell type (Hoyer et al. 1994). 5-HT2 receptors and other receptors are coupled with phospholipase C (Peroutka, 1993; Hoyer et al. 1994). The 5-HT3 receptor is the receptor channel coupled with ion movement (Riad et al. 1994) and is modulated by intracellular cyclic adenosine monophosphate (cyclic AMP) (Hoyer et al. 1994).

In the recent past, the importance of the fish brain monoaminergic system in aggression, mating and feeding has been documented. There are several apparent similarities between the functioning of the fish and mammalian monoaminergic systems. In fish, the hypermetabolism of catecholamines (norepinephrine and dopamine) and indoleamine (serotonin, 5-hydroxytryptamine, 5-HT) has been found to be associated with stressful conditions. In contrast to the situation in mammals, these monoamines can pass through the blood–brain barrier in teleost fishes, contributing to the high levels of biogenic amines in the periphery. Hence, high levels of serotonin in the peripheral circulation, during different stressful conditions, may influence the functioning of other physiological systems, notably the immune system. Serotonin is also stored in considerable amounts by mast cells and platelets, and can be synthetized by chromaffin cells. In mammals, it has been established that 5-HT modulates immune function at a variety of levels. However, little is known about the role of serotonin in the functioning of the immune system in fish. In this perspective article, we will discuss our results and the findings of other laboratories, although meagre on this subject, on the possible role of serotonin in the functioning of immunocompetent cells in fish.

Key words: serotonin, immunomodulation, rainbow trout, fish, stress, immunosuppression.
Stress induces hyperactivity of serotonin metabolism

Several investigators have demonstrated that stressful conditions induce hyperactivity in the metabolism of brain serotonin both in mammals (Khan, 1986; Khan and Hasan, 1984; Khan and Kalra, 1988) and in fish (McIntyre et al. 1979; Winberg and Nilsson, 1993). In fish, different kinds of stresses, e.g. exposure to a predator (Winberg et al. 1993), hierarchic dominance and agonistic behaviour, bring about an increase in brain serotonin activity (Winberg et al. 1991, 1992a,b). In Arctic charr Salvelinus alpinus, the 5-hydroxyindoleacetic acid (5-HIAA)/5-HT concentration ratio, an index of brain serotonin activity, is increased in certain areas of the brain of subordinate fishes (Winberg et al. 1992a). Hence, brain 5-HIAA/5-HT ratio and 5-HIAA concentrations are directly correlated with social stress in these species (Winberg et al. 1992a). This relationship between brain 5-HT utilization and social rank in teleost fish seems to be socially induced and not caused by innate differences in brain serotonin activity, predisposing fish for dominant or subordinate positions in a dominance hierarchy (Winberg and Nilsson, 1993). Exposure of damselfish Pomacentrus pontitus to a predator (a grayby) resulted in increases of several-fold in both 5-HIAA concentration and the 5-HIAA/5-HT ratio in the telencephalon, hypothalamus and brainstem (Winberg et al. 1993).

Recently, de Boeck et al. (1996) have shown that thermal and saline stress increase both the serotonin and dopamine contents in the hypothalamus of juvenile carp. It is noteworthy that the hypothalamus in fish brain is involved in the control of the physiological consequences of temperature. Increases in the hypothalamic serotonin content following thermal stress suggest that this biogenic amine may be directly involved in the regulation of temperature. Several investigators have pointed out that the increased serotonin activity observed under stressful conditions may be implicated in the regulation of endocrine function in fish (Burka et al. 1988; Khan and Thomas, 1992; Saligaut et al. 1992). In tilapia, serotonin has been found to stimulate prolactin secretion (Grau and Helms, 1990). Serotonin also controls the regulation of levels of cortisol, which plays an important role in salt-water adaptation in fish (Wendelaar Bonga, 1993). In mammals, it has been shown that there is a direct interaction between 5-HT and adrenocorticotropic hormone (ACTH): the former controls the secretion of the latter (Chaouloff, 1993). During stressful conditions in fish, ACTH secreted from the pituitary gland stimulates the synthesis and secretion of cortisol from the interrenal gland (Fagerlund, 1970).

**Immunomodulation by stress**

In fish, subpopulations of T and B lymphocytes have been identified and their functions have been reported to be similar to those of mammalian T and B cells (Clem et al. 1991; Miller et al. 1987; van Ginkel et al. 1994). Partula et al. (1994) have cloned the gene for the T cell receptor in rainbow trout (Oncorhynchus mykiss) lymphocytes. Roman et al. (1995) have cloned the gene for the immunoglobulin (the third complementary-determining region, CDRH3) that plays an important role in antigen recognition in B lymphocytes of rainbow trout. Accounts of the major histocompatibility complex (MHC) in fish are given in a recent review article (Stet et al. 1996). The cytokines, which are secreted by the immune cells and play an important role in cell signalling, have not been well characterized in fish species. Most effort has been directed towards biological assays of these agents rather than on their molecular characterization. The cytokines, like interleukin (IL)-1, IL-2 and macrophage activating factor, have been characterized biologically, and there is some cross-reactivity between fish cytokines and mammalian cytokines, demonstrating that these molecules exist in fish species. An exhaustive account of fish cytokines was given in a recent review article (Secombes et al. 1996).

It seems that, as in mammals, stress also exerts immunosuppressive effects in fish species. We have recently reported low antibody titres against Yersinia ruckeri in rainbow trout subjected to saline stress (Betoulle et al. 1995). Maule and Schreck (1990) concluded that the depressed antibody response in trout may be under hormonal control. These investigators demonstrated, for the first time, that fish lymphocytes express glucocorticoid receptors which might be implicated in immunosuppression during stressful conditions (Maule and Schreck, 1990, 1991). In a recent study, Maule et al. (1996) have demonstrated that high plasma cortisol titres are correlated with low numbers of antibody-producing cells (APC) in the chinook salmon (Oncorhynchus tsawytscha) and that, after acclimation to constant environmental conditions, plasma cortisol levels decrease concomitantly with an increase in numbers of APCs. Thermal stress has also been shown to influence fish immune function (Bly and Clem, 1992): low environmental temperature has been found to be immunosuppressive and to exert its effects on T, but not on B or accessory, cell function (Bly and Clem, 1992). The administration of small doses of cortisol and ACTH in fish can induce a decrease in the number of leucocytes, whereas large doses of ACTH given alone bring about leucocytosis (Saad, 1988). The effects of small doses of ACTH are similar to the effects of cold-induced stress: both induce a reduction in the number of circulating lymphocytes (Saad, 1988).

**Immunomodulation by serotonin**

Since stress has been shown to alter immune function (Harbuz and Lightman, 1992), a role for 5-HT in this immunomodulation has been proposed (Homo-Delarche and Durant, 1994), and several findings suggest that modulation of the immune system by serotonin occurs at the level of the lymphocytes (Aune et al. 1990, 1993). It is also noteworthy that 5-HT can be found at high concentrations in platelets, basophils, mast cells and gastrointestinal mucus (Jankovic, 1989). Moreover, mammalian lymphoid organs such as the spleen and thymus are innervated by serotonergic and catecholaminergic neurones, suggesting that leukocytes are directly exposed to 5-HT, even during the early stages of
Serotonin in fish immunomodulation

differentiation (Felten et al. 1991). Whether fish lymphoid organs are also innervated by monoaminergic neurones remains to be demonstrated. In mammals, the effects of 5-HT on the immune system have been reported to be either inhibitory or stimulatory, depending on the particular component of the immune system. In *in vitro* addition of exogenous serotonin has been found to suppress phytohaemagglutinin-induced blastogenesis (Khan et al. 1986). Serotonin has also been shown to augment interferon (IFN-γ)-induced phagocytosis (Sternberg et al. 1986) and natural killer cell cytotoxicity (Hellstrand and Hermodsson, 1987). Specific 5-HT receptors of the 5-HT1A receptor subtype have been identified and characterized on human T (Jurkat) cells and on mitogen-activated, but not resting, T cells (Aune et al. 1993). Serotonin stimulates T cell blastogenesis via these receptors by reducing intracellular cyclic AMP levels (Aune et al. 1993).

It is important to note that, in contrast to mammals, the biogenic amines can pass through the blood–brain barrier in teleost fish (Fritsche et al. 1993), possibly contributing to the high serotonin levels in the periphery during stressful conditions. It is also possible that the changes in peripheral circulating 5-HT levels could affect cerebral serotonergic activity; however, data are required to support this hypothesis. In mammals, there is a correlation between brain serotonin content and immunomodulation. The destruction of serotonergic neurones in rat brain has been found to result in low IgM and IgG antibody titres (Jackson et al. 1985). Furthermore, stressful conditions in the rat are known to increase levels of brain tryptophan (Dunn and Welch, 1991), the precursor of serotonin synthesis which can pass through the blood–brain barrier. It is important to mention here that the increases in brain serotonin levels in stressed fish are due to the release of serotonin from the nerve endings, not to the faster utilization of the tryptophan pool (Winberg and Nilsson, 1993); the polyamines abundantly present in trout brain may modulate the release of serotonin (Khan et al. 1996b). However, in mammals, the administration of exogenous tryptophan has been found to increase brain 5-HIAA concentrations (Fernstrom, 1987).

By analogy with mammals, it is possible that high levels of free 5-HT may influence the functioning of immunocompetent cells in fish. Hence, it is possible that circulating blood serotonin may exert an effect *via* specific membrane receptors present on lymphocytes (Fig. 1). We have previously shown that fish lymphocytes may possess receptors for different neuropeptides, such as substance P and somatostatin, which influence the proliferation of T and B cells (N’Doye et al. 1991, 1992). To our knowledge, there are no data available on the influence of 5-HT on the activation of the fish immune system. We have recently shown that serotonin exerts immunosuppressive effects on the lipopolysaccharide (LPS)- and phytohaemagglutinin (PHA)-stimulated proliferation of rainbow trout lymphocytes (Ferriere et al. 1996) and that, after mitogenic stimulation, 5-HT1A receptors are expressed on these cells. The 5-HT1B receptor subpopulation does not seem to be expressed either on resting or on mitogen-stimulated B and T lymphocyte subpopulations since a specific agonist of 5-HT1B receptors failed to influence significantly [3H] 5-HT binding to receptor sites. It is possible that the fish lymphocyte 5-HT1A receptor subtype is different from the mammalian receptor, as suggested by Heuring et al. (1986), and that there exist variations in brain 5-HT1A receptors in lower vertebrates (turtle, frog, chicken, etc.).

Since a number of studies have stressed the role of ion movements in the activation of mammalian immunocompetent cells (Chandy et al. 1985), it is possible that 5-HT3 receptor channels coupled with inward Na+ movements may influence the functioning of fish immune cells. Generally, the 5-HT3 receptor channel is coupled with inward Na+ and outward K+ transport in different cell systems (Bolanos et al. 1990). Choquet and Korn (1988) demonstrated for the first time the presence of K+ transport coupled with a 5-HT3 receptor.
channel in a mammalian pre-B cell line. We have identified the 5-HT$_3$ receptor on the lymphocytes of a teleost fish, Oncorhynchus mykiss (Meyniel et al. 1997). The pharmacological characteristics of this putative receptor are different from those of the mammalian receptor subtype: known antagonists of the mammalian 5-HT$_3$ receptor failed to displace $[^3]$H-serotonin bound to lymphocytes during association equilibrium. As for mammalian receptors, Na$^+$ also leaks into fish lymphocytes via the 5-HT$_3$ receptor channel (Meyniel et al. 1997). Serotonin and 2-methyl-5-HT exerted immunosuppressive effects via the 5-HT$_3$ receptor channel by blocking the progression of PHA-stimulated T cells through the cell cycle from the G$_0$/G$_1$ to the S phase. It is well documented that an increase in intracellular free Ca$^{2+}$ concentration, [Ca$^{2+}$], is an important mechanism for the transmission of cell surface activation signals coupled with diverse intracellular processes that culminate in cell proliferation and other functions (Berridge, 1993). The serotonin receptors are found to be coupled with Ca$^{2+}$ signalling in fish lymphocytes (Ferriere et al. 1997), as in mammalian immune cells (Khan et al. 1995). Serotonin mobilizes Ca$^{2+}$ via the 5-HT$_3$ receptor subtype by gating the Ca$^{2+}$ through L-type, but not N-type, Ca$^{2+}$ channels in rainbow trout lymphocytes (Ferriere et al. 1997).

Ishizuka et al. (1992) have demonstrated that, in pancreatic acinar cells, serotonin is synthesized de novo and regulates the growth of these cells via an autocrine loop. Besides intracellular synthesis, another source of serotonin will be the uptake by the cells to provide for their intracellular requirements may influence the functioning of immunocompetent cells. In support of our hypothesis, several studies have demonstrated that, in addition to neuronal cells, other non-excitable cells, such as platelets (Olson et al. 1974), endothelial cells (Myers et al. 1989), intestinal brush-border vesicles (Ramamoorthy et al. 1992) and human placental choriocarcinoma cells (Cool et al. 1981), express the serotonin transporter. Recently, we have shown that human lymphocytes possess a high-affinity serotonin transporter (Khan et al. 1996a).

In general, serotonin transport is a Na$^+$-driven phenomenon, regulated by protein kinase C, calmodulin and intracellular free [Ca$^{2+}$]. Fish lymphocytes also possess serotonin transport pathways (F. Ferriere et al., unpublished results). There are two possible mechanisms of action of transported serotonin: this biogenic amine may either act directly on its intracellular targets or perturb the second messenger cascades that are coupled with the antigen recognition sites (Deschaux and Khan, 1995). The preferred targets of internalized 5-HT will be either switching off its own intracellular biosynthesis or interacting with the Ca$^{2+}$/calmodulin complex, which activates calcineurin in order to translocate nuclear factors (e.g. NF-kB and STAT) from the cytoplasm to the nucleus during the course of immune cell activation. Alternatively, a ‘cross-talk’ between serotonin receptors and antigen recognition sites may operate and may be involved in the immunosuppressive effects of serotonin (Fig. 1). Hence, on the one hand, serotonin will activate the second messenger cascades via ligand–receptor interactions whereas, on the other hand, the antigen will transduce the signal to activate the immune cells. This ‘cross-talk’ may result in immunosuppression. Serotonin may also secondarily influence Ca$^{2+}$ signalling (see above) or the protein kinase C activation or/gene transcription involved in T and B cell activation. This overall disturbance in T or B lymphocyte activation may result in reduced secretion of cytokines and other soluble factors involved in cell–cell cooperation during an antigenic challenge. In addition, stressful conditions may also directly influence the lymphocyte membrane structures, e.g. by changing the distribution of serotonergic receptors. Alternatively, stress may induce a down-regulation of the second messengers, resulting in reduced activation of receptor-coupled protein kinase C or tyrosine kinase. This hypothesis is supported by our recent observation that saline stress (NaCl, 22%) reduced the number of lymphocyte serotonergic receptors in rainbow trout (Deschaux and Khan, 1996).

We conclude that a variety of stressful conditions may exert immunosuppressive effects in fish either via glucocorticoids or via an increase in the levels of biogenic amines, particularly of serotonin, which in turn will influence the functioning of immunocompetent cells via active transport systems or membrane receptors. Stress may also influence the number and density of lymphocyte receptors for these biogenic amines. The interaction between the neural, immune and endocrine systems is evident in fish because stress-induced increases in plasma cortisol concentrations are accompanied by the suppression of the immune response and reduced disease resistance (Maule et al. 1996). The overall immunosuppression of fish can affect their health and render them susceptible to opportunistic infections, resulting in economic consequences for the fishing industry.

References


REFERENCES


