Abstract

Evidence for the presence of a serotonin$_{1A}$ (5-HT$_{1A}$) receptor subtype in the salmonid fish brain has recently been presented. In the present study the potent 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) was tested for its effect on plasma cortisol concentrations in rainbow trout (Oncorhynchus mykiss). Blood was sampled and 8-OH-DPAT administered through a catheter in the dorsal aorta. Thirty minutes after the injection of 40 mg of 8-OH-DPAT/kg, plasma cortisol levels had increased from 12 to 149 ng/ml, whereupon they fell, reaching baseline levels after 4 h. The effect of 1–40 mg 8-OH-DPAT/kg on plasma cortisol concentrations was dose-dependent. The results lend further support to the hypothesis that the brain serotonergic system plays a key role in integrating autonomic, behavioral and neuroendocrine stress-responses in fish as well as mammals, suggesting that not only the structural and biochemical organization, but also the function of the serotonergic system has been conserved during vertebrate evolution. © 1997 Elsevier Science Ireland Ltd.

Keywords: Serotonin; Cortisol; Serotonin$_{1A}$ receptors; Rainbow trout; Stress; 8-Hydroxy-2-(di-n-propylamino)-tetralin

Numerous studies have presented evidence for a close relationship between the brain serotonergic system and the hypothalamic-pituitary-adrenal (HPA) axis in mammals. It appears clear that brain serotonin (5-hydroxytryptamine, 5-HT) synthesis and turnover is affected by stressful events [1,2,8,9]. Further, there are results suggesting that the sympathetic nervous system (SNS) as well as the HPA axis are involved in mediating stress-induced alterations in the 5-HT system of the mammalian brain (reviewed in Ref. [5]). Reciprocally, the serotonergic system appears to be involved in the control the HPA axis and SNS in mammals (reviewed in Refs. [5,7]). Thus, the brain 5-HT system could play a pivotal role in a complex neuroendocrine loop serving to defend homeostasis and promote acclimation during physiological or environmental challenges. Still, the role of brain 5-HT in HPA axis regulation has been debated, especially since direct 5-HT innervation of the mammalian paraventricular nucleus, the crucial focus for central regulation of the HPA axis, is limited [14]. However, direct synaptic contact between 5-HT nerve terminals and corticotropin-releasing hormone (CRH) containing neurons in the paraventricular nucleus has been demonstrated in the rat [16]. Moreover, a number of studies have shown that treatment with 5-HT precursors or 5-HT receptor agonists elevates plasma glucocorticoid levels in mammals, whereas 5-HT receptor antagonists or 5-HT synthesis inhibitors have the opposite effect [5,7].

The anatomical organization of the brain serotonergic systems is remarkably conserved among vertebrates [21,22], implicating that 5-HT functions might also have been conserved. For instance, there are results suggesting a relationship between the hypothalamic-pituitary-interrenal axis (HPI; the teleost homolog of the mammalian HPA axis) and the brain 5-HT system also in fish [31]. In a number of teleost species as well as in mammals, social subordination and other stressors, including handling and predator exposure, elevates brain 5-HT activity, as indicated by brain 5-hydroxyindoleacetic acid (5-HIAA; the major 5-HT metabolite) concentrations and 5-HIAA/5-HT ratios.
indoors in a holding tank continuously supplied with aerated trout. The rainbow trout used (400–500 g) were kept on plasma cortisol concentrations in catheterized rainbow trout achieved by studying the effect of 8-OH-DPAT injections like receptors are involved in HPI axis regulation in the Arctic charr (Salvelinus alpinus), whereas inhibition of 5-HT synthesis by p-chlorophenylalanine has the opposite effect [34]. However, brain 5-HT could also play an important role in the regulation of the HPI axis. Socially subordinate fish display elevated plasma cortisol levels, and increased interrenal cell sizes, suggesting a chronic activation of the HPI axis [19].

The 5-HT system of the mammalian brain has been suggested to stimulate the release of CRH which in turn activates corticotrophs of the pituitary [5,7]. Further, 5-HT has also been suggested to act directly at the corticotropes of the mammalian pituitary to stimulate the release of adrenocorticotropic hormone (ACTH) [5,7]. In mammals, the stimulatory role of 5-HT on the HPA axis has been attributed to 5-HT1A and 5-HT2 receptors [7]. Recently, Winberg and Nilsson [30] characterized three 5-HT receptor subtypes in the Arctic char brain. One of these receptors showed a pharmacological profile strikingly similar to the mammalian 5-HT1A receptor, as for example high affinity for 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) but low affinity to mianserin, a typical 5-HT2 receptor ligand [23].

The aim of the present study was to examine if 5-HT1A-like receptors are involved in HPI axis regulation in the rainbow trout (Oncorhynchus mykiss). This was to be achieved by studying the effect of 8-OH-DPAT injections on plasma cortisol concentrations in catheterized rainbow trout. The rainbow trout used (400–500 g) were kept indoors in a holding tank continuously supplied with aerated trout over time. At the start of the experiment (t = 0) a blood sample was taken and 8-OH-DPAT or saline (controls) were injected through the catheter. Subsequently blood samples were taken at 30, 60, 150 and 240 min after injection. Responses shown are mean ± SE.

As suggested in mammals [6,27], this elevation of brain 5-HT activity could mediate the stress-induced behavioral inhibition characterizing socially subordinate fish. In fact, pharmacological stimulation of brain 5-HT activity inhibits spontaneous locomotor activity and exploratory behavior in Arctic char (Salvelinus alpinus), whereas inhibition of 5-HT synthesis by p-chlorophenylalanine has the opposite effect [34]. However, brain 5-HT could also play an important role in the regulation of the HPI axis [19].

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The sensitivity of the standard curve ($r^2 = 0.964 ± 0.022$) was 0.92 ± 0.25 ng/ml, the intra-assay coefficient of variation (CV) was 2.1% and the inter-assay CV was 7.1%. Cortisol-free blanks gave a count of 1–2% of the total count.

Uppsala tap water (8–11°C) for more than 3 months before the experiment. The automatic light/dark regime which was continuously adjusted to conditions at latitude 51°N. In the holding tanks, the fish were fed daily with commercial trout pellets (EWOS ST40, Astra-EWOS Sweden) at 1–2% of the body weight. The fish were anesthetized (2-phenoxy-ethanol, 500 mg/l in the water) and catheterized in the dorsal aorta, as described by Soivio et al. [26], whereupon they were isolated in individual tanks and allowed to recover for 1 week. The catheter was attached to the outside of the tank, which was covered with black plastic. Moreover, the water surface was covered with floating plastic balls making the experimenter invisible to the fish, and thus allowing injections and sampling of blood without disturbance to the fish. The fish were not fed during the experiment.

8-OH-DPAT (obtained from Sigma Chemical Co.) was dissolved in saline at a concentration of 0.2 mg/ml. All experiments were performed between 1000 and 1400 h. At the start of the experiment a blood sample was taken and 8-OH-DPAT or saline (controls) were injected through the catheter. Subsequently, blood samples (100–200 μl) were taken through the catheter, using a heparinized syringe, at 30, 60, 150 and 240 min after injection. The sample volume was returned to the fish as saline. Blood samples were centrifuged immediately and plasma was removed and stored at −80°C.

Cortisol analysis was performed directly on rainbow trout plasma without extraction, using a radioimmunoassay described by Olsen et al. [20].

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The effects of 8-OH-DPAT (40 μg/kg) and saline on plasma cortisol concentrations over time are shown in Fig. 1. Administration of 8-OH-DPAT resulted in a rapid elevation of plasma cortisol concentrations. Thirty minutes after the injection of 40 μg of 8-OH-DPAT/kg, plasma cortisol levels had increased from 12 ± 5 to 149 ± 10 ng/ml (mean ± SE). Plasma cortisol concentrations subsequently decreased and were back at baseline levels after 4 h. Controls treated with an equal volume of saline showed constant plasma cortisol concentrations throughout the 4 h experiment. A repeated measure ANOVA showed that treatment (F(3,24) = 11.659, P = 0.0142) as well as time (F(4,24) = 11.864, P = 0.0008) had significant effects on plasma cortisol concentrations. Further, this test showed that there was a significant interaction between time and treatment (F(4,24) = 8.310, P = 0.0036).

The effects of saline and different doses of 8-OH-DPAT (1, 10 and 40 μg/kg) on plasma cortisol concentrations are summarized in Fig. 2. The experiment was performed as described above except that only two blood samples were taken; the first immediately before drug administration and the second 60 min after injection of 8-OH-DPAT, or saline. The effect of 8-OH-DPAT on plasma cortisol levels showed a clear dose-response relationship (effect of dose, F(3,15) = 16.408, P < 0.0001) and significant elevations in plasma cortisol concentrations were observed with 10 and 40 μg/kg of 8-OH-DPAT.

These results show that the specific 5-HT1A receptor agonist, 8-OH-DPAT, is a potent activator of the interrenal stress response in the rainbow trout. Furthermore, the time course of this effect, and the clear dose-response relationship, suggest that this is a specific effect, most likely mediated by a 5-HT1A-like receptor. 8-OH-DPAT is one of the most selective 5-HT1A agonists available. Winberg and Nilsson [30] found the Ki for 8-OH-DPAT at 5-HT1A-like receptors in the Arctic char brain to be 1.7 nM, which is within the range of Ki values reported for 8-OH-DPAT at mammalian 5-HT1A receptors [35].

The results of the present study are in good agreement with the results obtained in mammals. 8-OH-DPAT as well as other 5-HT1A agonists have repeatedly been found to elevate plasma glucocorticoid levels in mammals [3,13,29]. Furthermore, it appears well established that 5-HT stimulates CRH release from the mammalian hypothalamus and that 5-HT1A is the dominant receptor in mediating this effect [5,7]. The effect of 5-HT at the mammalian anterior pituitary has been less investigated. Still, there is a growing body of evidence suggesting that 5-HT also acts directly at the pituitary, stimulating the release of ACTH [7]. These pituitary effects of 5-HT have been attributed to 5-HT1A and 5-HT2 receptor activation [7]. Moreover, 5-HT has been reported to potentiate the effect of other ACTH secretagogues, such as arginine vasopressin (AVP), suggesting a possible amine-peptide interaction at the level of the pituitary [7].

Similarly, in the rainbow trout 8-OH-DPAT might act at different levels in the HPI axis. In the teleost brain, CRH containing neurons are found in the preoptic area and in the basal hypothalamus, two areas richly innervated by 5-HT fibers [12]. The pituitary pars distalis in teleosts is unique among vertebrates in that it is directly innervated by neurosecretory fibers and lack the portal system of the median eminence [24]. Indeed, the only way 5-HT originating from the central nervous system can reach the pituitary is through direct innervation. Such innervation has been detected in some teleost species [11,15,18] but Frankenhuysen van den Heuvel and Niwenhuys [12] could not demonstrate any structural relationship between brain 5-HT neurons and the pituitary gland in rainbow trout.

In addition to CRH several other neuropeptides/hormones are involved in the regulation of ACTH secretion from the teleost pituitary. For instance, arginine vasotocin (AVT), isotocin (IT) and urotensin I (UI) are all potent ACTH secretagogues [28]. AVT and IT are teleost homologs of mammalian AVP and oxytocin, respectively [28], whereas UI is a member of the CRH family of peptides [17]. 5-HT have been reported to stimulate the release of AVP [4] and oxytocin [25] in mammals but the role of 5-HT in the regulation of production and release of AVT and IT in teleost fish has not been studied.

In conclusion, the results from the present study show that 8-OH-DPAT elevates plasma cortisol in catetherized rainbow trout in a dose-dependent manner. The effect of 8-OH-DPAT is most likely mediated by activation of 5-HT1A-like receptors, 8-OH-DPAT being a highly selective 5-HT1A receptor agonist. This conclusion is further supported by the fact that previous results clearly show that 5-HT1A-like receptors are present in the brain of salmonid fish. However, at the present time, we do not know at which level of the HPI axis 8-OH-DPAT exerts its effect.

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