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Subclinical effects of saxitoxin and domoic acid on aggressive behaviour and monoaminergic turnover in rainbow trout (*Oncorhynchus mykiss*)

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The algal produced neurotoxins saxitoxin and domoic acid may have serious effects on marine life and can be responsible for the intoxication of for instance sea mammals, sea birds and fish. Given that farmed fish cannot escape algal blooms, they may be more susceptible to intoxication than wild stocks. In the present study, subclinical effects of saxitoxin and domoic on aggressive behaviour and monoaminergic systems in the brain of the rainbow trout (*Oncorhynchus mykiss*) were investigated. The resident-intruder test was used to measure aggression where only the resident fish were subjected to the toxins and analysed for monoamines and their metabolites. The resident-intruder test was carried out on two consecutive days. On day one basal aggression was measured in the four groups. On day two three of the groups were injected with subclinical doses of one of the following: saxitoxin (1.752 μg/kg bw), domoic (0.75 mg/kg bw) or 0.9% saline solution. This was performed 30 min prior to the aggression test. Handling stress and injection affected aggressive behaviour, cortisol and the serotonergic system in telencephalic brain regions. Cortisol levels were elevated in all of the injected groups when compared to the control group. An increase in serotonergic turnover was evident when all injected groups were pooled and compared to the control group. All together this suggests that the handling stress in connection with the injection was similar in all of the three injected groups. In contrast to both the undisturbed control group and the toxin-injected groups, the saline-injected group displayed a reduction in aggressive behaviour which was evident in increased attack latency. Furthermore the domoic injected group displayed more aggressive attacks towards their conspecifics than the saline-injected group. Consequently the two toxins appear to mask the stress induced alteration in aggressive behaviour. Monoamine levels and monoaminergic turnover could not be demonstrated to be directly affected by the two toxins at the given doses in the investigated brain regions (dorsal and ventral parts of telencephalon, optic tectum, locus coeruleus, raphe nucleus, molecular and granular layer of cerebellum). This could indicate that the toxins mediate aggressive behaviour either through other systems than the monoaminergic systems, such as neuroactive amino acids, or that the mediation occurs in other brain regions.

1. Introduction

Marine organisms are annually subjected to harmful algal blooms which can potentially cause serious intoxication of humans, sea birds and sea mammals via the food web. Reported effects on crustaceans, molluscs and finfish include mechanical damage and sometimes even mortality (for review, see Landsberg, 2002). Algae species in the *Alexandrium* family have been reported to be the cause of mortality in both wild fish (White, 1980), and farmed fish (Cembella et al., 2002). Toxicological symptoms occurring in milkfish (*Chanos chanos*) fingerlings subsequent to exposure to the *Alexandrium* cells comply with the symptoms observed following exposure to toxic extracts from the same algae (Chen and Chou, 2001). Moreover zebrafish (*Danio rerio*) larvae display both morphological and behavioural alterations ensuing exposure to the

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Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine, serotonin; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; ASP, amnesic shellfish poisoning; bw, body weight; Cg, granular layer of corpus cerebelli; Cm, molecular layer of corpus cerebelli; CNS, central nervous system; DA, dopamine; Dc, central zone of dorsal part of telencephalon; Dd/Dl, dorsal/lateral zone of dorsal part of telencephalon; Dm, medial zone of dorsal part of telencephalon; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, γ-aminobutyric acid; HCl, hydrochloric acid; HPLC-ED, high performance liquid chromatography with electrochemical detection; HVA, homovanillic acid; IP, intraperitoneal; LC, locus coeruleus; L-DOPA, L-3,4-dihydroxyphenylalanine; MCI, 95% confidence interval for the median; MHPG, 3-methoxy-4-hydroxyphenylglycol; NA, noradrenalin; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione; NMDA, N-methyl-D-aspartate; OT, optic tectum; PCA, perchloric acid; PSP, paralytic shellfish poisoning; R, superior raphe nucleus; Vv, ventral zone of ventral part of telencephalon.

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same toxin (Lefebvre et al., 2004). The toxins produced by this algae family are saxitoxin and its analogues, also known as paralytic shellfish poisoning (PSP) toxins. A further toxin producer is the *Pseudonitzschia* family which produces domoic acid, commonly known to cause amnesic shellfish poisoning (ASP). Northern anchovy (*Engraulis mordax*) has been shown to be the domoic acid vector of intoxication of sea birds (Work et al., 1993) and sea mammals (Lefebvre et al., 1999; Scholin et al., 2000), causing illness and sometimes even mortality. It has also been demonstrated that the anchovy itself displays neurotoxic symptoms after domoic acid exposure (Lefebvre et al., 2001).

Given that farmed fish are unable to escape an algal bloom, they may be more susceptible to intoxication than wild stocks. Huge economic losses due to mass mortalities in the fish farming industry are easy to calculate. However, the toxins may also cause sublethal effects including increased stress behaviour and loss of appetite, thereby reducing the overall growth of the fish (Landsberg, 2002). These effects are harder to translate into figures and therefore an understanding of the sublethal effects of ecotoxins on farmed fish species is of the utmost importance.

Domoic acid is known to cross the blood–brain barrier following systemic administration in mammals (Tryphonas et al., 1990) as well as in fish (Lefebvre et al., 2001). However, the effects following gastric administration may be less significant due to a high solubility in water (7.1 g/l at pH 3.1) and a low partitioning into lipids (octanol–water partition coefficient, K_{OW}: 0.0037 at pH 5.32) (Falk et al., 1991). Thus a low degree of absorption from the intestinal tract is expected (Hardy et al., 1995; Lefebvre et al., 2007). Saxitoxin is also a toxin with a high solubility in water and a low partitioning into lipids (octanol–water partition coefficient, K_{OW}: <0.001) (Llewellyn, 2006), thereby indicating a low degree of intestinal absorption. In salmon, the consequence of administering the toxin orally as opposed to systemically, is a 100 fold increase in LD_{50} (White, 1981). Saxitoxin is shown to cross the blood–brain barrier in mammals (Borison et al., 1980; Naseem, 1996) whereas the degree of transfer to the CNS in fish is to our knowledge unknown. However, effects on brain activity have been reported (Salierno et al., 2006; Bakke and Horsberg, 2007).

As both toxins affect the brain in fish behavioural effects can be expected. Previously described alterations in the general swimming pattern in domoic acid-exposed fish following systemic administration indicate an elevated state of activity. More specifically the pattern includes corkscrew swimming, circling, air gasping, swimming upside down and spiralling on the surface in a disoriented manner (Salierno, 2005; Bakke and Horsberg, 2007). The behavioural effect of saxitoxin in fish resembles the effects of an anaesthetic, i.e., a gradual paralysis and loss of equilibrium (White, 1981; Mortensen, 1985). Paralytic effects are previously reported in a variety of species including zebrafish (Lefebvre et al., 2004), herring (Lefebvre et al., 2005), killifish (Salierno et al., 2006) and salmon (White, 1984; Mortensen, 1985; Bakke and Horsberg, 2007). The behavioural alterations induced by domoic acid may be explained by its effect on non-NMDA glutamate receptors in the brain, imitating the effect of this excitatory amino acid. The behavioural effects of saxitoxin are a result of the toxin binding to voltage gated sodium channels in excitatory cells, resulting in a blocking of the inward Na^+ current while simultaneously leaving the outward K^+ current unaffected (Hille, 1975; Ritchie and Rogart, 1977; Cestele and Catterall, 2000). This results in hyperpolarization of the cell and subsequent paralysis when muscle cells are affected. As the compound may cross over to the brain, alterations in neuroactive amino acids release may also contribute to the symptoms (Cervantes et al., 2009).

The simplest way to measure changes in brain activity is to look at well established behavioural models. Aggressive behaviour is widely studied in salmonids like rainbow trout, with the resident-intruder test being frequently used as a model (Overli et al., 1999, 2002; Schjolden et al., 2005). Fish are isolated in interactive compartments in order to establish territorial safety prior to the test. When an intruder is introduced the resident fish will defend its "home tank" by displaying aggressive behaviour towards the intruder. In an attempt to encourage the resident fish to become aggressive the intruder is usually smaller than the resident fish. The latency to first attack and number of attacks between the two conspecifics is measured over a limited period of time. Aggressive behaviour has been coupled to monoaminergic turnover in several parts of the brain in fish (Winberg and Nilsson, 1993; Hoglund et al., 2005; Carpenter et al., 2009) as well as in lizards (Korzan et al., 2001, 2006; Summers et al., 2005), and rodents (Delville et al., 2000). In general long-term increases in serotonergic turnover is thought to depress aggression and the effects are modulated in specific regions. On the contrary short term stress, unavoidable during social interactions, is positively coupled to serotonergic turnover in other regions (for discussion, see (Summers and Winberg, 2006). Motivation for aggressive behaviour is, at least partly, controlled by the dopaminergic system (Korzan et al., 2006). Furthermore it has been shown that treatment with the synthetic dopamine precursor L-DOPA counteracts the elevated serotonergic turnover caused by stress (Hoglund et al., 2001). Thus elevation in dopaminergic turnover is generally related to increased aggression. The noradrenergic system may also be involved in the regulation of aggressive behaviour (Maler and Ellis, 1987). However stress coping (Adell et al., 1988) and responsiveness to novel environments (Sara et al., 1994) appear to be more dominant.

Both domoic acid (Arias et al., 1995) and saxitoxin (Cervantes et al., 2009) have been demonstrated to stimulate the serotonergic system in parts of the rat brain. Domoic acid has also been shown to increase the release of dopamine from synapses in the striatum of rats but decrease the metabolism of this monoamine (Quintela et al., 2000). It was therefore relevant to study the monoamine turnover in several regions of the rainbow trout brain.

The aim of the current study was to investigate if subclinical levels of saxitoxin and domoic acid could affect aggressive behaviour and monoaminergic turnover in different brain regions. In order to determine the effect of handling stress two control groups were included, one undisturbed control group and one saline-injected control group.

2. Material and methods

Rainbow trout (*Oncorhynchus mykiss*) were transported approximately 40 km from the University of Life Sciences (Ås, Norway) to the University of Oslo more than two weeks prior to the experiment. The fish were immediately divided into two groups according to size, one resident group and one intruder group.

The resident fish ($n = 32$, 153.6 ± 17.4 g) were isolated in compartments (50 cm × 24 cm × 43 cm) where four compartments constituted one experimental tank. Water was continuously supplied at one end of the experimental tank and released at the opposite end. The average temperature during the experiment was 8.7 °C (lowest 7.5 °C, highest 11 °C). The temperature in one compartment did not vary more than 0.5 °C throughout the entire duration of the experiment. In the course of a 14-day acclimatization period resident fish were fed individually with a commercial dry feed and any uneaten feed was removed. The resident fish were starved 3 days prior to the resident-intruder test and in the course of the 2-day experimental period,

Intruder fish (106.7 ± 12.4 g) were kept in a large tank with a consistent water flow and a constant temperature of 7.5 °C. They were fed daily with commercial dry feed. Prior to the resident-intruder tests the intruders were weighed in order to match the resident fish.