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Sex differences in effects of low level domoic acid exposure

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ABSTRACT

Consumption of seafood containing the phytoplankton-derived toxin domoic acid (DOM) causes neurotoxicity in humans and in animals. It has been reported that DOM-induced symptoms may be more severe in men than women, but to date the effect of sex on DOM-induced effects in adults is not known. We investigated sex differences in DOM-induced effects in adult rats. Since low level exposure is of greatest relevance to human health (due to DOM regulatory limit), we examined the effects of low level exposure. Adult male and female Sprague Dawley rats were administered a single intraperitoneal injection of DOM (0, 1.0, 1.8 mg/kg). Behaviour was monitored for 3 h and immunohistochemistry in the dorsal hippocampus and olfactory bulb was also examined. DOM increased locomotor and grooming activity, compared to vehicle group. DOM exposure also significantly increased stereotypic behaviours and decreased phosphorylated cAMP response element-binding protein immunoreactivity (pCREB-IR). There was no effect of sex on the magnitude of the behavioural responses, but the onset of DOM-induced locomotor activity and ear scratches was quicker in females than in males. Mixed effect modelling revealed the predicted peak in locomotor activity in response to DOM was also quicker in females than in males. Severe toxicity was evident in 2/7 male rats and 0/8 female rats dosed with 1.8 mg/kg DOM. These data suggest that males exposed to low level DOM may be more susceptible to severe neurotoxicity, whereas females are affected more quickly. Understanding sex differences in DOM-induced neurotoxicity may contribute to future protective strategies and treatments.

1. Introduction

Consumption of seafood containing the phytoplankton-derived neurotoxin domoic acid (DOM) can cause the clinical syndrome "amnesic shellfish poisoning" (ASP) in humans (Perl et al., 1990). Exposed individuals experience gastrointestinal and neurological symptoms including confusion, disorientation, memory loss and seizures, with histopathological abnormalities evident at post-mortem (Perl et al., 1990; Teitelbaum et al., 1990). Similar neurological symptoms and fatalities have been reported in sea animal populations exposed to DOM (Lefebvre et al., 2010; Silvagni et al., 2005; Work et al., 1993), indicating the neurotoxic mechanism and response to DOM is conserved across species. DOM, which is structurally related to kainic acid, acts via an excitotoxic mechanism, whereby activation of non-NMDA receptors leads to excessive endogenous glutamate release (Berman and Murray, 1997; Debonnel et al., 1989; Larm et al., 1997). Given that recent data suggest that the frequency and magnitude of DOM-producing algal blooms are increasing (Sekula-Wood et al., 2011) and that the number of reports of shellfish and animal populations exposed to DOM continues to increase (Hall and Frame, 2010; James et al., 2005; Ujevic et al., 2010), DOM remains a threat to human and animal health. Therefore, understanding DOM neurotoxicity continues to be of importance.

During an incident of human DOM exposure in Canada in 1987, 107 people were diagnosed with ASP (Perl et al., 1990). Interestingly, significant differences were evident between males and females. Men were reported to be more likely to have memory loss and require hospitalisation (Perl et al., 1990). In toxicology, sex can influence exposure opportunity, toxicokinetics and toxicodynamics leading to sex-specific toxic responses (Vahter et al., 2007).

Understanding sex differences in the response to neurotoxins is of great importance for adequate risk assessment strategies to protect both males and females (Vahter et al., 2007). However, to date only one study has specifically examined the effect of sex on DOM-induced neurotoxicity in adults (Wetmore and Nance, 1991).

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DOM injection into the lateral septal area of rats was reported to cause more cell loss in males than females. Taken together with the evidence from the Canadian outbreak, the results from this study indicate that there may be a sex difference in susceptibility to DOM neurotoxicity in adults.

Under laboratory conditions, DOM has been shown to produce a reliable series of behavioural changes in rodents, within a few hours of systemic exposure, that are (a) reproducible (b) dose dependent and (c) time dependent (time of onset is inversely proportional to dose) (Tasker et al., 1991; Tryphonas et al., 1990). Low doses of 1–2 mg/kg given via intraperitoneal (i.p.) injection (and high doses soon after exposure) produce changes in activity, progressing to stereotypic behaviours, for example, ear scratching and wetdog shakes (Tasker et al., 1991; Tryphonas et al., 1990). High doses of 4 mg/kg i.p. or above produce status epilepticus, for example, continuous tremor, convulsions, and death. DOM exposure also produces histological changes in the brain. Low doses have been reported to alter the expression of proteins involved in glutamate receptor-mediated signal transduction pathways (Qiu and Currás-Collazo, 2006). High doses, associated with convulsions, cause neuronal cell loss and astrogliosis predominantly in the hippocampus and olfactory bulb (Colman et al., 2005; Peng et al., 1994; Pulido, 2008; Tryphonas et al., 1990). These studies indicate that lower, sub-convulsive doses of DOM can produce reproducible and quantifiable effects in rodents, indicating it is unnecessary to expose animals to high convulsive-inducing doses in order to assess DOM neurotoxicity. Furthermore, since the introduction of a regulatory limit (20 μg DOM/g shellfish tissue; estimated to produce half the no-observed-adverse-effect-level (NOAEL) see (Kumar et al., 2009)) for harvested shellfish, as a result of the 1987 Canadian outbreak of DOM intoxication (Wekell et al., 2004), most humans have been protected from high level DOM exposure. Therefore, consequences of low level exposure are of greatest relevance to human health (see Lefebvre and Robertson, 2010).

The objective of the current study was to further understand of the consequences of low level DOM exposure and determine if there is a sex difference in susceptibility to DOM neurotoxicity in adults, which could aid future risk assessment strategies. To assess DOM neurotoxicity, we exposed adult male and female rats to sub-convulsive doses of DOM; 1.0 mg/kg, which was expected to alter activity without stereotypic behaviours (1.5 times the NOAEL in rats) and 1.8 mg/kg, which was expected to alter activity and produce stereotypic behaviours without convulsions (2.8 times the NOAEL in rats (see Kumar et al., 2009)). We then evaluated quantifiable behavioural and immunohistochemical changes, that have been shown to be reliably produced by DOM exposure in previous studies (Colman et al., 2005; Peng et al., 1994; Qiu and Currás-Collazo, 2006; Tasker et al., 1991; Tryphonas et al., 1990). Behavioural measures were locomotor activity, grooming, ear scratching and wetdog shakes. The impacts of DOM exposure on behavioural responses was then investigated using Generalised Mixed Effect Modelling (GLMM). Immunohistochemical measures were the expression of glial fibrillary acidic protein (GFAP), a marker of gliosis, and phosphorylated cyclic adenosine 3′5′ monophosphate-response element binding protein (pCREB), a protein activated in many cellular pathways including glutamate receptor-mediated signal transduction, in the dorsal hippocampus and olfactory bulb.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act of 1986 and the European Community Council Directive of 24 November 1986 (86/609/EEC). Adult male and female Sprague Dawley rats from Charles River (Kent, UK), aged between 14 and 18 weeks, were housed in same sex groups of four in a temperature controlled room (21 ± 2 °C) with 12:12 h light/dark cycle (lights on at 07:00) with free access to RM3 rat chow (Special diets service, Edinburgh, UK) and water. Daily vaginal lavages were collected from females and estrous cycle stage was determined by cytological evaluation of methylene blue stained lavage fluid on glass slides.

2.2. Treatments

After a minimum 5 day acclimatisation period, age matched male and female rats were randomly assigned to a treatment group, 0 (8 male, 8 female), 1.0 (9 male, 8 female), or 1.8 mg/kg (7 male, 8 female) DOM (Tocris, Bristol, UK). Rats were administered vehicle or 1.0 or 1.8 mg/kg DOM dissolved in distilled water vehicle (dose volume 1 ml/kg) via a single i.p. injection, to replicate the exposure route used in previous DOM studies (Colman et al., 2005; Kuhlmann and Guilarte, 1997; Peng and Ramsdell, 1996; Peng et al., 1994; Tasker et al., 1991; Tryphonas et al., 1990). All female rats were dosed on the day of proestrus. Proestrus was selected as this is when circulating progesterone levels peak, and so may be when sex differences are maximal. One male rat (1.8 mg/kg) displayed convulsions shortly after exposure so was humanely killed and not included in the behavioural analysis. Another male rat (1.8 mg/kg) had a poor prognosis the day after exposure so was humanely killed. Neither rat was transcardially perfused, so were not included in the histological analysis.

2.3. Behavioural analysis

Immediately after treatment, rats were placed into individual Opto-Varimex 3 activity chambers with clear transparent walls (43 cm × 43 cm × 30 cm: Columbus Instruments, Ohio, USA) equipped with two rows of infra-red photocells 5 cm from the floor, for 180 min. Each chamber was connected to a computer that converted beam breaks to distance travelled (cm). The number of periods the rats spent engaged in an individual behaviour, for example, grooming, ear scratching or wetdog shakes, in a 2 min-on and 3 min-off cycle, was recorded while the rats were in the activity chambers for 180 min.

2.4. Tissue preparation

Our pilot studies demonstrated that DOM-induced GFAP effects were not evident 1 day after DOM exposure but were evident 3 days after. Therefore, three days after DOM exposure, rats were deeply anesthetised with pentobarbital and perfused transcardially with chilled 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4). Brains were removed, cut into four coronal slices and post-fixed in the same fixative overnight at 4 °C before being cryoprotected in 10%, 20% and then 30% sucrose in 0.1 M PBS at 4 °C. Slices were rapidly frozen on a metal block in a freezer and stored at −80 °C until cut. Slices containing the dorsal hippocampus were cut on a cryostat into 20 μm thick sections between Bregma −3.8 and −4.16 mm (Paxinos and Watson, 1998) and thaw mounted onto VECTABOND™ coated slides. Three sections per slide were collected. Slices containing the olfactory bulb were halved sagittally and the left bulbs cut into 20 μm thick sections between Lateral 0.9 and 1.9 mm (Paxinos and Watson, 1998). Four sections per slide were collected. Slides were stored at −80 °C.

2.5. Immunohistochemistry

Hippocampal and olfactory bulb sections were post-fixed in 4% formalin in 0.1 M PBS for 10 min. Sections for pCREB