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Antinociceptive effects of buprenorphine in zebrafish larvae: An alternative for rodent models to study pain and nociception?

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ABSTRACT

The underlying processes of nociception and pain are, despite the rodent models available, still not fully understood. One of the drawbacks of rodent model systems is the difficulty to screen compound libraries for their influence on nociception, thus slowing down the discovery of novel analgesics for clinical use.

Rodent behavioural tasks have been previously adapted for larval zebrafish in our group and in the current manuscript we investigated the possibilities of zebrafish larvae as an additional model system to study nociception and pain and their underlying mechanisms.

Zebrafish larvae were exposed to different concentrations of diluted acetic acid, a chemical noxious stimulus, and we measured nociceptive-specific behaviours. Cyclooxygenase-2 (cox-2), a gene known to be involved in nociception, was used as a marker for the activation of nociceptive pathways. Upon exposure to diluted acetic acid, five-day old larval zebrafish showed a concentration dependent increase in locomotor activity. This increase in locomotor activity was accompanied by a stimulus dependent increase in cox-2 mRNA expression, demonstrating that nociceptive pathways were indeed activated. Pre-treatment of the larvae with 0.1 μg/ml buprenorphine before exposure to the noxious stimulus, prevented the behavioural changes induced by the diluted acid. Further, the antinociceptive properties of buprenorphine could be reversed by co-treatment with the μ-receptor antagonist naloxone.

In conclusion, our results demonstrate that larval zebrafish as young as five days, show behavioural responses upon exposure to a noxious stimulus. The magnitude of the responses is dependent on the intensity of the stimulus applied and activation of nociceptive pathways was confirmed by altered cox-2 mRNA expression. The analgesic buprenorphine has similar antinociceptive properties in this model as in higher vertebrates and mammals and is able to prevent the behavioural responses induced by the noxious stimulus. We therefore propose zebrafish larvae as a novel model system in nociception and pain related research.

1. Introduction

Nociception, the activation of nociceptive pathways by noxious or unpleasant stimuli, is a phenomenon closely related to pain. By far, most research on pain and nociception has been performed in mammalian model systems. The number of studies in non-mammalian species is

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limited (see Newby et al., 2009, for an overview) and there is no commercially available analgesic for fish while the use of zebrafish in many research fields is increasing rapidly. Recently, it has been shown that several species of fish can be used as a model system to study the effects of noxious stimuli (Braithwaite and Boulcott, 2007; Chervova and Lapshin, 2000; Correia et al., 2011; Gonzalez-Nunez and Rodríguez, 2009; Mettam et al., 2011; Reilly et al., 2008b; Sneddon, 2003b; Stevens, 2008b; Weber, 2011; Yue, 2009). Fish possess the physiological and neuro-anatomical structures required for nociceptive responses similar to mammals (Sneddon, 2003a, 2006; Stevens, 2008a). In trout for example, the presence of functional nociceptin receptors in the head region and skin has been described (Ashley et al., 2007; Sneddon, 2003a; Sneddon et al., 2003) and the nociceptin receptors of zebrafish have been cloned (Sanchez-Simon and Rodriguez, 2009; Rivas-Boyero et al., 2011) offering the possibility for future functional analysis of nociceptive pathways. The existence of these receptors in fish highlights a conserved gene pool during evolution with possible conserved functions. Most studies in fish use injection of diluted acetic acid as noxious stimulus and the resulting behaviours are fully described (Correia et al., 2011; Maximino, 2011; Mettam et al., 2011; Newby and Stevens, 2008; Reilly et al., 2008a; Sneddon, 2003b; Sneddon et al., 2003).

Recently the first papers have been published using adult zebrafish as a model (Correia et al., 2011; Gonzalez-Nunez and Rodríguez, 2009; Maximino, 2011; Reilly et al., 2008b), but the use of larvae in particular, as we show for the first time in the present study, could be a powerful tool in the field of nociception and pain research. Larval zebrafish are used in a wide range of research fields and their rapid development, transparency and cheap maintenance opens possibilities to perform large scale screens for new compounds with analgesic properties as for example snake venom or plant extract protein databases. In vivo imaging and morpholino-based gene knock-down are valuable technologies to gain a better insight into molecular and cellular processes underlying nociception in zebrafish larvae.

Our aim was to develop a behavioural model that displays specific nociceptive responses and which is easy to apply in 96-well screening assays thereby reducing the amount of experiments performed on higher vertebrates. To demonstrate that diluted acetic acid is indeed activating nociceptive pathways, we measured the expression of a gene known to be involved in the processing of noxious stimuli. Upon damage of peripheral tissues, prostaglandins are released and activate primary afferents and sensitize nociceptors (Sinatra, 2002). Cyclooxygenase-2 (cox-2), is a major enzyme for prostaglandins production (Chen et al., 2012) and several studies showed an increase in the expression of this enzyme upon tissue damage in acute and chronic pain in inflammation models (Chen et al., 2012; Ebersberger et al., 1999; Hay and De Belleroche, 1997; Lee et al., 2005; Samad et al., 2001; Sinatra, 2002; Zhao et al., 2000).

In the present study, different concentrations of acetic acid were used to assess the behavioural repertoire and the changes in gene expression associated with nociception in larval zebrafish. Indeed, it has been previously reported that low pH is one of the greatest stressors for fish as shown by laboratory data or in the wild (Åtland, 1998; Kroon, 2005; Lacoul et al., 2011). Besides the hydrogen ion toxicity, it has been elegantly demonstrated that acidic water triggers clear behavioural changes of the fish such as avoidance and antipredator behaviour (Brown et al., 2012; Leduc et al., 2004). To minimize the discomfort of the larvae, the lowest effective concentration was chosen for further experiments. Buprenorphine, a post-operative morphine-like analgesic used in mammals and humans, and the antagonist naloxone were used for validation. In all experiments conducted, a control group which underwent the same handling procedure in absence of the noxious stimulus was included too as a control for handling stress (Maximino, 2011; Roques et al., 2012).

2. Materials and methods

2.1. Statement of ethic on animal use

All experimental procedures were conducted in accordance with The Netherlands Experiments on Animals Act that serves as the implementation of “Guidelines on the protection of experimental animals” by the Council of Europe (1986), Directive 86/609/EC, and were performed only after a positive recommendation of the Animal Experiments Committee had been issued to the license holder.

2.2. Animal husbandry

Male and female adult zebrafish (*Danio rerio*) of AB wild type were purchased from Selecta Aquarium Speciaalzaak (Leiden, The Netherlands). Fish were kept at a maximum density of 12 individuals in plastic 7.5 L tanks (1145, Tecniplast, Germany) containing a plastic plant as tank enrichment, in a zebrafish recirculation system (Fleuren & Nooijen, Nederweert, The Netherlands) on a 14 h light:10 h dark cycle (lights on at 7 h AM/lights off at 21 h PM). Water and air temperature were maintained at 24 °C and 23 °C, respectively. Fish were purchased at the juvenile stage and were allowed to adapt to our facility for at least 2 months before being used as adult breeders. The fish were fed twice daily, once with dry food (DuplaRin M, Gelsdorf, Germany) and once with frozen brine shrimp (Dutch Select Food, Aquadistri BV, The Netherlands) according to standard procedures. Zebrafish eggs were obtained by random mating between sexually mature individuals.

Eggs were harvested the morning after mesh nets were applied to the adult tanks and age of the embryos was set as days post fertilization (dpf) 1. This is based on the staging system employed in Zebrafish: practical approach (Nusslein-Volhard and Dahm, 2005). Approximately 100 eggs were transferred in 10 cm Petri dishes filled with egg water (0.21 g/L Instant Ocean Sea Salt and 0.0005% (v/v) methyl blue) and kept in a separate climate room maintained at a temperature of 28 °C and 30% humidity and under a light–dark cycle of 14 h:10 h (lights on at 7 h AM/lights off at 21 h PM). At the age of 3 dpf, 25 embryos were transferred to a well of a 12-well plate containing