Scale Up Isolation of Aaptamine for In Vivo Evaluation Indicates Its Neurobiological Activity is Linked to the Delta Opioid Receptor

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Scale up isolation of aaptamine for in vivo evaluation indicates its neurobiological activity is linked to the delta opioid receptor

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Introduction

Opioid receptors belong to the large superfamily of seven transmembrane-spanning (7TM) G protein-coupled receptors (GPCRs). As a class, GPCRs are of fundamental physiological importance mediating the actions of the majority of known neurotransmitters and hormones. The Mu, Delta and Kappa (MOR, DOR, KOR) opioid receptors are particularly intriguing members of this receptor family as they are the targets involved in many neurobiological diseases such as addiction, pain, stress, anxiety, and depression. To date few marine natural products have been investigated for their neurobiological activities.3 One noteworthy example involves ziconotide (1) from the cone snail Conus magnus.2 Compound 1 was the first marine natural product approved by the FDA and is used for the treatment of pain, marketed under the trade name Prialt® (2004).3 More recently Hamman reported that aaptamine (2) is the first marine natural product to show in vivo antidepressant activity, however no mechanism of action was proposed.4 During a separate collaborative screening project we profiled 96 sponge-derived extracts and discovered demethyl-aaptamine (3) and demethyl (oxy) – aaptamine (4) were selective DOR agonists as shown in Figure 1. We speculated that the in vivo activity for 2 could thus be linked to the DOR target and to test this hypothesis we conducted the following experiments below.

Experimental and Results

Our first step involved obtaining a source of aaptamine (2) for in vitro and in vivo evaluation. Compounds 3-4 were obtained from the sponge Aaptos aaptos (coll. no. 92553) but were devoid of 2. LC-MS analysis of sponge coll. no. 11308 (A. aaptos) indicated m/z ions of 229.1 amu consistent with that of 2 (not shown). We extracted coll. no. 11308 using a partition scheme shown in Figure 2. The WB extract was enriched with 90% MeOH/H2O MeOH/H2O (138.8 mg) and used to scale up its isolation by HPLC shown in Figure 3a. The LC-MS data in Figure 3b) was confirmatory. aaptamine (2) was effective in WT but not DOR knock out (KO) mice (* p<.05 compared to saline).

Conclusions

1) Scale up isolation of aaptamine (2) is best achieved through purification of water soluble extracts. 2) The mechanism of action for the in vivo anti-depressant-like and anxiolytic-like activity of 2 is mediated by it’s activity on the delta opioid receptor (DOR). 3) These data suggest that 2 can represent a novel chemical scaffold for the development of new DOR ligands in neurobiological research.

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References


Figure 1. a) LC-MS library with annotations including m/z ions and b) comparative DOP agonist activity of the methanol extract LC fractions of coll. no. 92553 FM.

Figure 2. Extraction Scheme Flowchart of coll. no. 11308

Figure 3. a) Analytical traces LC (top) MS (bottom) of coll. no. 11308 WB and b) Preparative scale up HPLC traces of coll. no. 11308 WB fractions. Gradient: 10% → 100% CH3CN (45 min); [4.0 mg/100 µl] x 30 injections; λmax = 254 nm; sensitivity = 2.0 AU; flow 2.0 min/ul.

Figure 4. Chemical validation of aaptamine (2) using: a) LC-MS-ELSD analysis with annotations including m/z ions and b) 1H NMR data of 11308 WB H6.

Figure 5. Mice were injected with saline or aaptamine (2, 40mg/kg, i.p.) and subjected to: a) a forced swim test (a), a locomotor test (b) or a marble burying test (c).

Swiss strains litter 10m (in ambient light of 500-600lx) and were conducted in individual clear plastic cylinders (17.5cm tall × 25cm in diameter) filled with water (25°C, 40°C) to a depth of 20cm. A camera positioned on an angle to the cylinders recorded the sessions. Forced swimming was for a total invariable time during the last 30s of the session. Immobility was defined as the absence of movement, except that necessary to keep afloat. Following the test, mice were placed in a locomotor chamber to assess general locomotion for 30 minutes. For the marble burying test, mice were placed in a cage with 5 cm of wood shavings and 33 evenly spaced marbles for 20 minutes. At the end of the session, the number of marbles buried and exposed were counted. aaptamine (2) was effective in WT but not DOR knock out (KO) mice (* p<.05 compared to saline).