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# Isodomoic acids A and C exhibit low KA receptor affinity and reduced *in vitro* potency relative to domoic acid in region CA1 of rat hippocampus ☆

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**Abstract**

Several natural isomers of the seizurogenic neurotoxin domoic acid (DA) have been found to occur at up to mg/kg levels in shellfish. The aim of the current study was to assess the neurotoxic potency of isodomoic acids A and C (Iso-A and Iso-C), recently isolated from commercial shellfish. Hippocampal slices were obtained from young adult rats and maintained in a tissue recording chamber. Synaptically evoked population spikes were recorded in region CA1 before and after exposure to DA or its isomers. Both Iso-A and Iso-C produced transient neuronal hyperexcitability followed by a dose-dependent suppression of population spikes, but were, respectively, 4- and 20-fold less potent than DA (spike area: $EC_{50}$ DA = 237 nM; Iso-A = 939 nM; Iso-C = 4.6 μM). In the hippocampus, DA preconditioning induces tolerance to subsequent DA toxicity. However, in the present study neither Iso-A nor Iso-C were effective as preconditioning agents. Competitive binding studies using homomeric GluR6 kainate (kainic acid, KA) receptors showed the affinity of Iso-A to be 40-fold lower than DA ($K_i$ DA = 3.35 nM; Iso-A = 130 nM). Together with earlier work showing Iso-C affinity at GluR6 receptors to be 240-fold lower than DA, our results suggest that neuroexcitatory effects of Iso-A in CA1 may involve both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and KA receptors, while Iso-C likely involves the activation of AMPA receptors alone.


*Keywords:* Domoic acid; Isodomoic acid A; Isodomoic acid C; KA receptor affinity; Hippocampal slice; *In vitro* preconditioning

## 1. Introduction

Domoic acid (DA) is a potent agonist of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainic acid (KA)-sensitive ionotropic glutamate receptors (Lerma et al., 1993; Tasker et al., 1996; Larm et al., 1997; Hampson and Manolo, 1998; Crawford et al., 2000; Sari and Kerr,

☆ *Ethical statement*: All procedures were approved by the University of Otago Animal Ethics Committee, in accordance with the New Zealand Animal Welfare Act, and in accordance with guidelines for the ethical and humane use of animals in research, established under the UK Animals (Scientific Procedures) Act 1986 (Chapter 14, Schedule 1, Table A).

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2001; Jakobsen et al., 2002; Qiu and Currás-Collazo, 2006; Qiu et al., 2006) and has been identified as a causative agent in cases of animal and human poisoning (Perl et al., 1990; Teitelbuam et al., 1990; Lefebvre et al., 1999; Scholin et al., 2000; Gulland et al., 2002). Acutely, activation of AMPA/KA receptors by DA produces neuronal depolarization, hyperexcitability and severe generalized motor seizures, which are particularly robust in aged animals (Hesp et al., 2004a). In addition, DA can produce substantial excitotoxic lesions of the hippocampus and related limbic circuits, and has been associated with lasting neurological deficits including chronic epileptiform activity and amnesia in humans (Perl et al., 1990; Cendes et al., 1995).

DA is produced by marine red algae in the genus *Chondria* and diatoms in the genus *Pseudo-nitzschia*. Previous work has shown that in addition to DA, a number of natural isomers also exist (isodomoic acid (Iso) A through H; Wright et al., 1990a; Zaman et al., 1997; Zhao et al., 1997; Campbell et al., 2004; Clayden et al., 2005; Kotaki et al., 2005). Radioligand-binding studies using rat forebrain membranes revealed Iso-D, Iso-E and Iso-F to have substantially lower affinities for both KA and AMPA receptors relative to DA (Hampson et al., 1992). Recently, Iso-C was found in unprecedented concentrations in New Zealand shellfish (Rhodes et al., 2004) and its affinity for homomeric GluR6 KA receptors was shown to be 240-fold lower than DA (Holland et al., 2005). Iso-A (along with Iso-C) has also been detected at similar concentrations in New Zealand scallops (Selwood et al., unpublished observations). Toxicity profiles for several DA isomers have been established in insects and in these studies isomers A, B, C and F were found to be 10–20-fold less potent, and Iso-D and Iso-E were over 100-fold less potent than DA (Maeda et al., 1984, 1986). Similar findings were noted in an early study of functional toxicity by isomers D, E and F in cultured mouse neurons (Wright et al., 1990b). However, to date the electrophysiological properties of DA isomers have not been investigated in intact mammalian CNS tissue preparations.

The *in vitro* hippocampal slice preparation has proved effective in examining the functional pharmacology of various neurotoxins (Kerr et al., 1999). In hippocampal region CA1, DA characteristically produces transient hyperexcitability followed by a dose-dependent suppression of synaptically evoked field potentials (Kerr et al., 1999; Sari and Kerr, 2001). Interestingly, hippocampal slices that undergo prior exposure to DA are subsequently resistant to the neuroexcitatory effects of the toxin, even at relatively high *in vitro* concentrations (Kerr et al., 2002; Hesp et al., 2004b). This "preconditioning" effect may constitute an important endogenous neuroprotective mechanism. The aim of the current study was to assess the functional *in vitro* pharmacology of Iso-A and Iso-C in this well-defined mammalian CNS preparation. The seizurogenic potency of each isomer was assessed relative to the parent molecule, and their effectiveness as preconditioning agents was assessed in hippocampal CA1 *in vitro*. In addition, following earlier work with Iso-C (Holland et al., 2005), radioligand-binding studies were carried out to assess the affinity of Iso-A relative to DA at homomeric GluR6 KA receptors.

## 2. Materials and methods

### 2.1. Chemicals and tissue preparation

DA was purchased from Sapphire Bioscience PTY. Ltd. (NSW, Australia). All other compounds were of reagent grade and purchased from B.D.H. Laboratory Supplies (Poole, UK). Iso-A and Iso-C (Fig. 1A) were obtained from the Cawthron Institute, Nelson, New Zealand. Isolation and purification of isomers from digestive glands of contaminated scallop tissues were carried out according to established procedures (Holland et al., 2005; Selwood et al., unpublished observations). Briefly, tissues were extracted with aqueous methanol, the filtered extract was washed with chloroform and the methanol removed by rotary evaporation. DA and isomers were isolated using Strata-X sorbant (Phenomenex, CA) and purified using column and preparative high-performance liquid chromatography on C18 phases. The separated isomers were $>98\%$ pure by LC-UV and LC-MS. Concentrations of DA, Iso-A and Iso-C used in the testing were determined by LC-MS (SIR) using calibration against certified reference standards of DA and Iso-C (CRMP-NRCC, Halifax, NS).

All animal procedures were conducted under approval of the University of Otago Animal Ethics Committee. Young (2–3 months) male Wistar rats were anesthetized with $CO_2$, and sacrificed by rapid decapitation. Brains were removed to ice-cold artificial cerebrospinal fluid (ACSF) consisting of 124 mM NaCl, 2.5 mM $CaCl_2$, 3.2 mM KCl, 1.3 mM $MgCl_2$, 1.25 mM $NaH_2PO_4$, 26 mM $NaHCO_3$ and 10 mM glucose, saturated with 95% $O_2$/5% $CO_2$.