# Epi-inositol is ineffective in Porsolt Forced Swim Test model of depression

The six-carbon polyol inositol is a uniquely versatile compound in biology. Myo-inositol is the major naturally occurring isomer of nine possible inositol isomers and is critical for the phosphoinositide (PI) cycle as a substrate for PI synthase (Berridge and Irvine 1989). Benjamins and Agranoff (1969) reported that epi-inositol is not a substrate for PI synthase, but a number of experiments suggest that epi-inositol might affect the PI cycle. Like myo-inositol, epi-inositol was shown to reverse Li-pilocarpine seizures (Williams and Jope 1995), strongly suggesting that epi-inositol could enter the PI cycle. Furthermore, accumulation of [3H]-cytidine monophosphate phosphatidate (CMP-PA) in Chinese hamster ovary (CHO) cells during Li treatment is reversible with myo-inositol, and Richards and Belmaker (1996) found that epi-inositol was about 30%–40% as active as myo-inositol in reversing such accumulation. Myo-inositol has been found to have antidepressant and antianxiety effects in animal models (Einat and Belmaker 2001) and in controlled studies in patients (Levine 1997). Recently, Einat et al (1998) studied epi-inositol in the elevated plus maze model of rat anxiety and surprisingly found it much more active than myo-inositol.

In this report, we explored the effects of epi-inositol in the Porsolt Forced Swim Test model of depression (FST) and have attempted to replicate our previous results in the Elevated Plus Maze.

For the FST, rats (n = 10/group) were tested with two exposures to a water tank spaced 24 hours apart. Epi-inositol treatment (5 g/kg diluted in deionized water to 20 mL/kg) consisted of two injections, the first immediately after the first exposure to the water tank and the second 5 hours prior to the second exposure (the test session). This dose and schedule of injections was chosen because it was demonstrated to be effective in this model with myo-inositol (Einat et al 1999, 2001). The Elevated Plus-Maze model for anxiety study was designed to replicate our previously reported experiment (Einat et al 1998) and included 3 groups (n = 10/group) receiving chronic (11 days) daily intraperitoneal injections at a dose of 1.2 g/kg myo-inositol, epi-inositol, and control solutions. Five to six hours after the last injection, animals were tested in an elevated plus-maze. A student's t-test was used to analyze the FST results. Since data were not homogenous (Levene test: $F(2,26) = 3.8$, $p < 0.04$), the Plus-Maze measures were analyzed using a Kruskall-Wallis nonparametric analysis of variance (ANOVA) followed by similar post-hoc comparisons of open/closed arms entries and time ratio.

Epi-inositol treatment did not affect immobility ($t(18) = 0.4$, NS), swimming ($t(18) = 0.84$, NS), or struggle time ($t(18) = 0.24$, NS) in the FST. As previously reported, myo-inositol had an anxiolytic-like effect in the plus-maze model as demonstrated by significant increase in open/closed arms entries ratio (Kruskall-Wallis ANOVA, $\chi^2(2) = 7.2$, $p < 0.03$, post hoc tests myo-inositol different than control) and a similar trend for increase in open/closed arms time ratio ($\chi^2(2) = 5.6$, $p = 0.06$). However, in contrast with our previous findings, epi-inositol had no anxiolytic effects in the elevated plus-maze model.

Epi-inositol was not active in the FST, and the previous finding that epi-inositol is more effective than myo-inositol in the elevated plus maze model of anxiety was not replicated. Effects of myo-inositol in the FST have been shown to be mediated by the serotonergic system, specifically via 5-HT2A/C receptors (Einat et al 2001). Epi-inositol is not a substrate for PI synthase, is not incorporated into phosphatidylinositol, and does not enter into the PI cycle second messenger system (Benjamins and Agranoff 1969). The present finding therefore supports the hypothesis that myo-inositol's antidepressant and anxiolytic effectiveness is based on its ability to affect the PI cycle activity.

Epi-inositol, similar to myo-inositol, was active in the reversing of biochemical and behavioral effects of lithium action (Godfrey 1989; Patishi et al 1996), and in yeast, epi-inositol was shown to affect the expression of genes involved in the metabolism of myo-inositol (Shaldubina et al 2002). Therefore, the effect of epi-inositol, which is not incorporated into the PI cycle, in biochemical and behavioral models of lithium action could be explained by an epi-inositol effect on myo-inositol metabolism at the level of gene expression. However, as demonstrated by the present results, epi-inositol may not be enough to independently induce behavioral change in standard models of depression or anxiety at the same design where myo-inositol induces a clear and replicable behavioral change.

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