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RAPID COMMUNICATION

AR-A014418, a selective GSK-3 inhibitor, produces antidepressant-like effects in the forced swim test

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Abstract

The mechanism by which lithium exerts either its anti-manic or antidepressant effects remains to be fully elucidated. Although lithium inhibits the enzyme glycogen synthase kinase-3 (GSK-3) at concentrations that are relevant for treatment of bipolar disorder, it is unclear whether GSK-3-related mechanisms are responsible for its therapeutic effects in the treatment of this disease. We report that AR-A014418 (a selective GSK-3 inhibitor) induces behavioural changes that are consistent with the effects of antidepressant medications. Subacute intraperitoneal injections of AR-A014418 reduced immobility time in rats exposed to the forced swim test, a well-established model for antidepressant efficacy. In addition, the specificity of this effect is supported by our finding that AR-A014418 decreased spontaneous as well as amphetamine-induced activity. Taken together, these data support the hypothesis that lithium may exert its antidepressant effects through inhibition of GSK-3, and that novel small-molecule GSK-3 inhibitors may be useful for the treatment of bipolar disorder and depression.

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Introduction

Lithium exerts both anti-manic and antidepressant actions (collectively mood stabilization) when used clinically. Although widespread in its clinical use worldwide for three decades, the therapeutic mechanism(s) of action of this monovalent cation remains unknown. More than 1% of the world's population suffers from severe bipolar disorder, and up to 15% suffers from severe depression. It is anticipated that understanding the therapeutic target of lithium will allow for both an improved understanding of the pathophysiology of this disorder, and for – using a hypothesis-driven approach – the ability to treat patients with medications having greater specificity, increased efficacy and fewer side-effects.

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At therapeutic concentrations, lithium inhibits a small number of enzymes via competition for magnesium. In mammals, this group includes at least four related phosphomonoesterases (the best known of which is inositol monophosphatase) and the metabolic enzyme phosphoglucomutase (see Gould et al., 2004b for review). Recent evidence indicates that among several kinases, lithium specifically inhibits glycogen synthase kinase-3 (GSK-3) at therapeutic concentrations in vitro (Klein and Melton, 1996), and in the rat brain (Gould et al., 2004a). However, it is unclear if this is relevant for its therapeutic effects in the treatment of bipolar disorder. AR-A014418 has recently been described as a selective GSK-3 inhibitor, which acts in an ATP competitive manner (Bhat et al., 2003). It does not significantly inhibit 26 other kinases (Bhat et al., 2003). In the present study, we sought to determine if pharmacological inhibition of GSK-3 with AR-A014418 has behavioural effects in animal models that are consistent with the mood-stabilization properties of lithium. Our results are consistent with the notion that pharmacological inhibition of GSK-3 could be an effective therapy for bipolar disorder and depression.

Methods

Animals

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA), weighing 200–230 g at the beginning of the experiments were housed, two per cage, in an animal room with constant temperature (22 ± 1 °C) and a 12 h light/dark cycle, with free access to food and water. All experimental procedures were approved by the Animal Use Committee of the National Institute of Mental Health (Protocol number LMP-07-02) and were conducted according to NIH guidelines.

Drugs

The GSK-3 inhibitor AR-A014418 (30 μmol/kg; obtained from AstraZeneca R&D Södertälje, Sweden) was dissolved in DMSO and injected (1 ml/kg i.p.). AR-A014418 readily passes the blood–brain barrier following peripheral administration, and has an i.v. half-life of 1.1 h. Administration of 30 μmol/kg results in approx. 1.2 μM brain concentrations [GSK-3 K_i 38 nM (Bhat et al., 2003; R. Bhat, unpublished data)]. Amphetamine sulphate (Sigma, St. Louis, MO, USA; 0.5 mg/kg) was dissolved in saline and injected i.p. (1 ml/kg).

Spontaneous and amphetamine-induced activity

A large open field (120×120 cm) was utilized to study spontaneous activity and a smaller arena (40×40 cm) was utilized to study amphetamine-induced activity. Thirty minutes after AR-A014418 (or vehicle) injection and immediately after amphetamine injection (in the amphetamine experiment) rats were placed in the centre of the arena and their behaviour recorded for 45 min to the Ethovision videotracking system (Noldus, Leesburg, VA, USA) and to videotapes.

Forced swim test

The forced swim test (FST) involved two exposures to a cylindrical tank of water (23 ± 1 °C) where rats cannot touch the bottom of the tank or escape. For the first exposure, the rats were placed in the water for 10 min. Twenty-four hours later, the rats were placed in the water again for a 5-min session (test session; for detailed description of the procedure see Einat et al., 2001). Rats were injected with AR-A014418 or vehicle twice, immediately after the first exposure to the tank and 30 min prior to the test exposure. Behaviour was videotaped for later analysis and the periods of immobility were scored from videotapes.

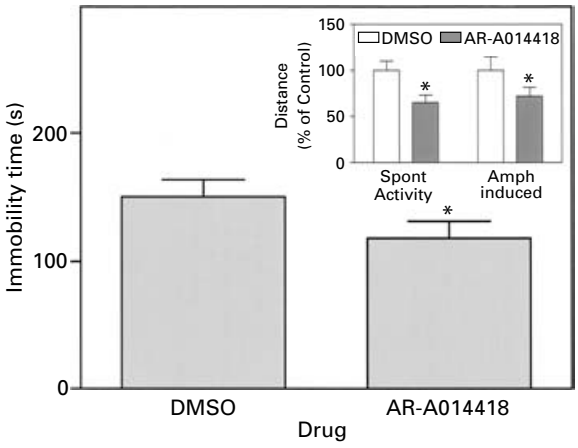

Figure 1. Immobility time in the forced swim test (in seconds); $n = 14$ per group; bar graphs represent mean ± S.E. Rats treated with AR-A014418 demonstrated significantly reduced immobility time compared to vehicle-treated animals. *Inset*: Spontaneous (left bars) and amphetamine-induced (right bars) activity shown as percent of control. $n = 7$ and 6 per group for spontaneous and amphetamine-induced activity respectively; bar graphs represent mean ± S.E. AR-A014418-treated animals (shaded bars) show significantly reduced activity in both tests compared to vehicle rats (open bars). * Signifies $p < 0.05$.

Statistical analysis

Since all tests were conducted in replicas (for technical reasons), data were analysed using a 2-way ANOVA with drug treatment as one factor and replica as a second factor. Level of significance was set at $p < 0.05$.

Results

Forced swim test

Rats treated with AR-A014418 demonstrated significantly reduced immobility time compared to control animals in the FST, suggesting an antidepressant-like action [Figure 1; ANOVA drug effect $F(1, 22) = 5.2$, $p < 0.05$].

Activity

Treatment with AR-A014418 resulted in reduced spontaneous and amphetamine-induced activity supporting the specificity of the antidepressant-like effect in the FST [Figure 1, inset; spontaneous activity: ANOVA $F(1, 10) = 9.8$, $p = 0.01$; amphetamine-induced activity: ANOVA, drug effect $F(1, 8) = 14.8$, $p < 0.01$].

Discussion

AR-A014418 reduces immobility time in rats when tested in the FST, and the specificity of these results is supported by a reduction in spontaneous and amphetamine-induced activity levels. The present data support a hypothesis that lithium may exert its antidepressant effects through inhibition of GSK-3, and that novel small-molecule GSK-3 inhibitors may be useful for the treatment of bipolar disorder and depression (Gould et al., 2004b; Kaidanovich-Beilin et al., 2004).

Kaidanovich-Beilin and colleagues recently reported that a peptide inhibitor of GSK-3 (L803-mts) produces antidepressant-like effects in the FST following intracerebral ventricle injections in mice (Kaidanovich-Beilin et al., 2004). Our data support and extend their data, showing that similar antidepressant-like effects are observed following pharmacological inhibition of GSK-3 in another species (rats), a different route of administration (peripheral), and by inhibiting GSK-3 via an alternative mechanism (L803-mts competes for the active site while AR-A014418 is ATP competitive). Furthermore, our study demonstrates that the effects of GSK-3 inhibition in the FST are specific to antidepressant-like effects and are not the consequence of a generalized increase in activity as both spontaneous and amphetamine-induced activity were reduced following AR-A014418 administration.

Although GSK-3 has a number of cytoplasmic and nuclear targets (see Frame and Cohen, 2001 for discussion), it is unclear whether the antidepressant-like effect we observe in the FST originates from a single or multiple substrate effects. However, the acuteness of the effects argues against sizable plastic changes playing a major role. In this regard, Li and colleagues recently reported that inhibitory phosphorylation of GSK-3 is acutely regulated by serotonin levels (Li et al., In Press). Specifically, when administered to mice, d-fenfluramine (serotonin release stimulator), clorgyline (MAOI), fluoxetine, and imipramine all increase serine 9 phosphorylation of GSK-3β (Li et al., In Press). Combined with the present results this finding raises the possibility that the effects of antidepressant drugs on rodent behavioural measures may be mediated by GSK-3-dependent mechanisms. GSK-3 inhibition may, therefore, represent a therapeutically relevant downstream consequence of antidepressant drugs that act via modulation of monamine levels. Antidepressant-like effects of lithium in the FST are inconsistent. However, in regard to the degree of GSK-3 inhibition, the dose of lithium that can be safely administered to rodents is much smaller that that of AR-A014418. This observation raises the possibility that if higher inhibition of GSK-3 could be clinically achieved by novel GSK-3 inhibitors, a more robust antidepressant effect may be possible.

Amphetamine-induced hyperactivity is the best established rodent model for mania and this hyperactivity is attenuated by a number of mood stabilizers including lithium, anticonvulsants, and antipsychotics (see Einat et al., 2000 for a review of these data). Beaulieu and colleagues recently reported that dopamine-dependent activity increases in mice are mediated in large part via a GSK-3-dependent mechanism (Beaulieu et al., 2004). They report that both lithium and alternate GSK-3 inhibitors attenuate the hyperactivity in mice lacking the dopamine transporter, that amphetamine administration to wild-type mice results in a decrease in the inhibitory phosphorylation of GSK-3, and that mice heterozygous for GSK-3β have an attenuated response to amphetamine administration (Beaulieu et al., 2004). Our findings of decreased amphetamine-induced hyperactivity in rats following AR-A014418 administration are consistent with these findings and further support the hypothesis that lithium's ability to attenuate amphetamine-induced hyperactivity may also be due to inhibition of GSK-3. In toto, these data support the possibility that, in addition to an antidepressant target, inhibition of GSK-3 may represent lithium's anti-manic target as well. Clearly additional work will be necessary prior to attributing such a 'bimodal action' to GSK-3 inhibition.

Future studies will be required to delineate precisely the mechanism by which inhibition of GSK-3 results in reduced FST immobility. Likewise, of critical importance will be a determination of what GSK-3 downstream target(s) may be the key molecular entities responsible for an antidepressant-like effect in this model. Clinically, lithium only exerts its full effects following weeks of treatment. It will thus be essential to determine the behavioural effects of long-term administration of AR-A014418. GSK-3 inhibitors are actively under development by the pharmaceutical industry and academia. It is anticipated that these will undergo trials for the treatment of bipolar disorder and depression.

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Statement of Interest

Ratan Bhat is employed by AstraZeneca.

References

Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, Gainetdinov RR, Caron MG (2004). Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proceedings of the National Academy of Science USA 101*, 5099–5104.

Bhat RV, Xue Y, Berg S, Hellberg S, Ormo M, Nilsson Y, Radesater AC, Jerning E, Markgren PO, Borgegard T, Nylof M, Gimenez-Cassina A, Hernandez F, Lucas JJ, Diaz-Nido J, Avila J (2003). Structural insights and biological effects of glycogen synthase kinase 3 specific inhibitor AR-A014418. *Journal of Biological Chemistry 278*, 45937–45045.

Einat H, Clenet F, Shaldubina A, Belmaker RH, Bourin M (2001). The antidepressant activity of inositol in the forced swim test involves 5-HT(2) receptors. *Behavioral Brain Research 118*, 77–83.

Einat H, Kofman O, Belmaker RH (2000). Animal models of bipolar disorder: from a single episode to progressive cycling models. In: Myslobodsky M, Weiner I (Eds.), *Contemporary Issues in Modeling Psychopathology* (pp. 165–179). London: Kluwer Academic Publishers.

Frame S, Cohen P (2001). GSK3 takes centre stage more than 20 years after its discovery. *Biochemistry Journal 359*, 1–16.

Gould TD, Chen G, Manji HK (2004a). In vivo evidence in the brain for lithium inhibition of glycogen synthase kinase-3. *Neuropsychopharmacology 29*, 32–38.

Gould TD, Zarate CA, Manji HK (2004b). Glycogen synthase kinase-3: a target for novel bipolar disorder treatments. *Journal of Clinical Psychiatry 65*, 10–21.

Kaidanovich-Beilin O, Milman A, Weizman A, Pick CG, Eldar-Finkelman H (2004). Rapid antidepressive-like activity of specific glycogen synthase kinase-3 inhibitor and its effect on beta-catenin in mouse hippocampus. *Biological Psychiatry 55*, 781–784.

Klein PS, Melton DA (1996). A molecular mechanism for the effect of lithium on development. *Proceedings of the National Academy of Science USA 93*, 8455–8459.

Li X, Zhu W, Roh MS, Friedman AB, Rosborough K, Jope RS (In Press). In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. *Neuropsychopharmacology*.