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Short communication

Antidepressant-like effect of chromium chloride in the mouse forced swim test: involvement of glutamatergic and serotonergic receptors

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Abstract:
Chromium (Cr) (III), an essential microelement of living organisms, was reported to exhibit potential antidepressant properties in preclinical and clinical studies. The aim of the present study was to examine the effect of $CrCl_3$ *ip* administration in the forced swim test (FST) in mice and the involvement of glutamatergic and serotonergic receptors in the antidepressant-like activity of chromium. $CrCl_3$ in a dose of 12 mg/kg, but not in doses of 6 or 32 mg/kg, reduced the immobility time in the FST. The locomotor activity was reduced by $CrCl_3$ in a dose of 32 mg/kg. Moreover, the reduction of the immobility time induced by the active dose (12 mg/kg) of $CrCl_3$ was completely abolished by NBQX (10 mg/kg; an antagonist of the AMPA receptor) pretreatment and partially inhibited by ritanserin (4 mg/kg; an antagonist of 5-$HT_{2A/C}$ receptor), WAY 1006335 (0.1 mg/kg; an antagonist of 5-HT_{1A} receptor) and N-methyl-D-aspartate (75 mg/kg; agonist of NMDA receptor) administration. The present study demonstrates the antidepressant-like activity of chromium in the mouse FST and indicates the major role of the AMPA receptor and participation of NMDA glutamatergic and 5-HT_{1A} and 5-$HT_{2A/C}$ serotonin receptors in this activity.

Key words:
chromium (III) chloride, NMDA, AMPA, 5-HT_{1A}, 5-$HT_{2A/C}$, receptors, forced swim test, mice

Introduction

Chromium (Cr) is an element that can occur in nine oxidation states (from Cr(-II) to Cr(VI)). Cr(III) is an essential microelement of living organisms, while Cr(VI) is highly toxic (carcinogenic, allergic, causing asthma, cardiovascular and renal disorders) [13, 14, 22]. Biotransformation of carbohydrates and lipids depends on the presence of Cr(III), thus its deficiency participates in the development of diabetes [13]. On the other hand Cr(III) supplementation improves glucose tolerance (insulin sensitivity) in diabetes [1, 13].

Co-morbidity between diabetes and depression is very high, with diabetic patients having twice as high a risk for depression than those who are non-diabetic. This combination also increases the mortality risk by over 36% [15, 17]. The antidepressant activity of chromium salts in affective disorders was reported [7, 10, 19–21]. Furthermore, some preclinical data illustrated the putative antidepressant activity of Cr and the involvement of serotonin in this action [3, 16]. The serotonin hypothesis of depression and antidepressant action points to the role of serotonin receptors in these mechanisms (see [6] for review). Recently, the glutamate system and in particular the role of the N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors in depression and antidepressant treatment was demonstrated (reviewed in [23, 24, 30]).

The aim of the present study was: 1) to evaluate the activity of chromium (III) chloride in the forced swim test (FST) in mice and 2) to examine the role of the glutamatergic NMDA and AMPA and the serotonergic 5-HT_{1A} and 5-$HT_{2A/C}$ receptors in the antidepressant effect evoked by Cr treatment.

Materials and Methods

Animals

All procedures were conducted according to the National Institute of Health Animal Care and Use Committee guidelines and were approved by the Ethical Committee of the Collegium Medicum, Jagiellonian University, Kraków. The experiments were carried out on male Albino Swiss mice (25–30 g). The animals were kept under a natural day-night cycle with free access to food and water.

Drug administration

Chromium (III) chloride ($CrCl_3$, Sigma-Aldrich, USA) and imipramine (30 mg/kg, Polfa, Poland) were administrated intraperitoneally (*ip*) 45 min before the test. N-methyl-D-aspartic acid (NMDA, 75 mg/kg, Sigma, USA), 2,3-dihydroxy-6-nitro-7-sulfoamoylbenzo(f)-quinozaline (NBQX, 10 mg/kg, Tocris, UK) were dissolved in 0.9% NaCl, ritanserin (RIT, 4 mg/kg, Tocris, UK) was dissolved in DMSO/0.9% NaCl (1:250) and administered *ip* 1 h before the test. WAY 1006335 (WAY, 0.1 mg/kg, synthesized by Dr. J. Boksa, Institute of Pharmacology, PAS, Kraków, Poland) was dissolved in 0.9% NaCl and administered subcutaneously. Control animals received the appropriate vehicle. All solutions were administered at a volume of 10 ml/kg.

Forced swim test

The studies were carried out on mice according to the method of Porsolt and co-workers [28]. Mice were individually propped in glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water, maintained at 23–25°C. The animals were left in the cylinder for 6 min. After the first 2 min, the total duration of immobility was measured during a 4 min test. The mouse was judged to be immobile when it remained floating passively in the water.

Locomotor activity

The locomotor activity of the mice was measured with photoresistor actometers (circular cages, diameter 25 cm, two light beams). The animals were individually placed in an actometer for 2 min. Activity was then measured for 4 min. The number of crossings of the light beams by the mice was then recorded as the locomotor activity.

Statistics

The data obtained were evaluated by the one-way analysis of variance (ANOVA), followed by Bonferroni's Multiple Comparison Test. All the results are presented as the mean ± SEM; $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Chromium picolinate salt was effective as an antidepressant treatment during a typical depression [7, 10]. Moreover, the adjuvant activity of Cr to antidepressant therapy was also reported in endogenous or dysthymic affective disorders [19, 20]. Thus, these preliminary studies suggest the antidepressant potential of Cr in affective disorders [21]. The preclinical

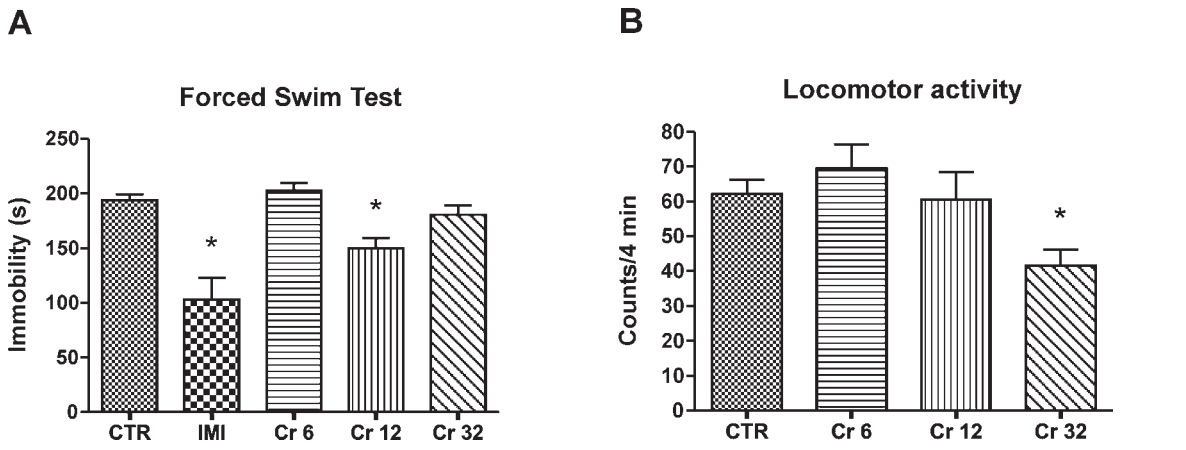

Fig. 1. **(A)** The effects of $CrCl_3$ (Cr) or imipramine (IMI) treatment on the total duration of immobility in the FST in mice. $CrCl_3$ or IMI were administered 45 min before the test. The values represent the mean ± SEM (n = 9–10 mice per group). ANOVA: $F(3, 35) = 19.64$, $p < 0.0001$. **(B)** The effects of $CrCl_3$ (Cr) treatment on the locomotor activity in mice. $CrCl_3$ was administered 45 min before the test. The values represent the mean ± SEM (n = 5–6 mice per group). ANOVA: $F(3, 18) = 4.342$, $p = 0.0181$. * $p < 0.05$ *vs.* control (CTR) group (Bonferroni test)

data on the evaluation of the antidepressant activity of Cr is also very limited. Khanam and Pillai [16] demonstrated the antidepressant-like effect of chromium picolinate in the modified rat FST.

The present study evaluates the effect of $CrCl_3$ in the mouse FST, which is shown in Figure 1A. $CrCl_3$ treatment at a dose of 12 mg/kg (but not in doses of 6 or 32 mg/kg) or imipramine (30 mg/kg, internal experimental standard) significantly reduced the immobility time in the FST in mice (by 22 and 47%, respectively). The effect of $CrCl_3$ on locomotor activity is shown in Figure 1B. $CrCl_3$ treatment at a dose of 32 mg/kg significantly reduced (by 33%) the locomotor activity, while the other doses (6 and 12 mg/kg) were ineffective by this measure. This indicates that $CrCl_3$ also exhibits antidepressant activity in the mouse FST. The lack of activity at the highest examined dose (32 mg/kg) in the FST was probably caused by a concomitant reduction in locomotor activity (Fig. 1B).

The authors mentioned above [16] not only demonstrated the antidepressant-like activity of Cr, but also indicated the involvement of the serotonin system in this effect. They observed Cr-induced reduction in immobility and enhancement in swimming, but no alteration in climbing behavior [16]. Additionally, since glimepiride (K^+ channel antagonist) potentiates the chromium effect on the swimming time, these authors [16] suggest the involvement of that particular channel in the antidepressant activity of Cr. Our objection to this notion comes from the lack of such potentiation in the immobility time, which is the most important parameter indicating antidepressant activity in this test [9, 28]. Returning to the involvement of the serotonin system in Cr antidepressant activity, there is more evidence to support this. Human and animal studies by Attenburrow et al. [3] and Franklin and Odontiadis [12] demonstrated an increase in the serotonin level and metabolism, plus the reduced sensitivity of the serotonin 5-HT_{2A} receptors following chromium picolinate administration.

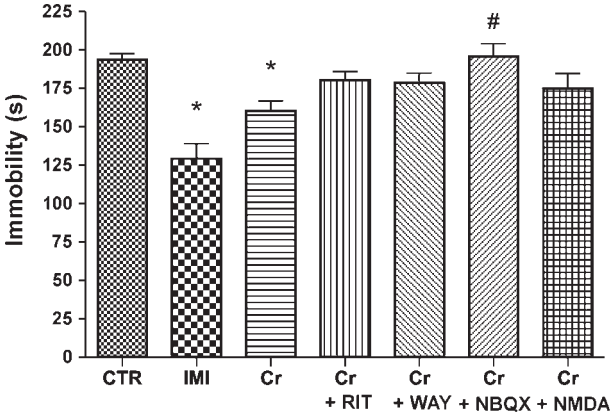

Fig. 2. The effects of $CrCl_3$ (Cr) treatment in combination with ritanserin (RIT), WAY 100635 (WAY), NBQX or NMDA on the total duration of immobility in the FST in mice. $CrCl_3$ or IMI were administered 45 min before the test. RIT, WAY, NBQX or NMDA were administered 1 h before the test. The values represent the mean ± SEM (n = 7–15 mice per group). ANOVA: $F(6, 59) = 10.74$, $p < 0.0001$. * $p < 0.001$ *vs.* control (CTR) group; # $p < 0.01$ *vs.* Cr group (Bonferroni test)

In the present study, we also measured the effect of pre-treatment with ritanserin (RIT, an antagonist of the 5-$HT_{2A/C}$ receptor), WAY 1006335 (WAY, an antagonist of the 5-HT_{1A} receptor), NBQX (an antagonist of the AMPA receptor), and NMDA (an agonist of the NMDA receptor) on the effect produced by $CrCl_3$ in the FST in mice, and the results are shown in Figure 2. $CrCl_3$ treatment (12 mg/kg) or imipramine (30 mg/kg, internal experimental standard) significantly reduced the immobility time in the FST in mice (by 20 and 33%, respectively). Administration of RIT (4 mg/kg), WAY (0.1 mg/kg), NBQX (10 mg/kg), or NMDA (75 mg/kg) alone had no effect on the immobility time (data not shown, but performed with another set of experiments being prepared for publication). However, NBQX completely (by 24%) and RIT (by 15%), WAY (14%), and NMDA (by 11%) partially antagonized the effect elicited by $CrCl_3$ in this test (Fig. 2). $CrCl_3$ in combination with the receptor ligands did not influence the locomotor activity (data not shown). Thus, in the present study we demonstrated the involvement of glutamatergic AMPA and NMDA receptors and serotonergic 5-HT_{1A} and 5-$HT_{2A/C}$ receptors in the antidepressant activity of $CrCl_3$.

The current serotonin hypothesis of depression and the mechanism of antidepressant action implicate the role of serotonin receptors in this concept (see [6] for review). The importance of 5-HT_{1A} receptor-mediated signaling in antidepressant-like activity in the FST was demonstrated [2, 9], while antagonism, but not enhancement, of the 5-$HT_{2A/C}$ receptors seems to have an antidepressant effect in both the rodent screening tests [8, 29] and clinical studies [5]. The present studies indicate that $CrCl_3$ antidepressant-like activity in the FST is dependent on serotonergic transmission *via* 5-HT_{1A} and 5-$HT_{2A/C}$, and therefore, chromium displays a 5-HT_{1A} profile similar to the classic antidepressants. The importance of the 5-$HT_{2A/C}$ receptors in this activity remains to be established.

Functional antagonists of the NMDA receptor complex exhibit antidepressant-like activity in the rodent test and models of depression (see [24, 31–34] for review). In previous studies, we demonstrated that the antidepressant-like effect of CGP 37849 (antagonist of the glutamate/NMDA receptor site) and L-701,324 (antagonist of the glycine/NMDA receptor site) was abolished by NMDA co-treatment [27]. Additionally, the involvement of the NMDA receptor in the antidepressant-like activity of inorganic NMDA antagonists such as magnesium and zinc was demonstrated [25, 26]. These indicate the crucial role of the NMDA receptor in the antidepressant-like action of NMDA antagonists in the FST. Such a mechanism was now demonstrated in the effect of $CrCl_3$.

The antidepressant activity of AMPA potentiators was demonstrated previously (see [4, 30] for review), and moreover, the dependence of the antidepressant-like effects of NMDA antagonists on AMPA signaling was also examined [11, 18]. Complete antagonism of the chromium-induced antidepressant-like effect by NBQX and partial antagonism by NMDA indicate involvement of the glutamate system in the $CrCl_3$ antidepressant activity.

In summary, the present study demonstrates the antidepressant-like activity of Cr in the mouse FST and indicates the major role of the AMPA receptor and participation of NMDA glutamatergic and 5-HT_{1A} and 5-$HT_{2A/C}$ serotonin receptors in this activity.

References:

1. Abdourahman A, Edwards JG: Chromium supplementation improves glucose tolerance in diabetic Goto-Kakizati rats. IUBMB Life, 2008, 60, 541–548.
2. Abert PR, Lemonde S: 5-HT_{1A} receptors, gene repression, and depression: guilt by association. Neuroscientist, 2004, 10, 575–593.
3. Attenburrow M-J, Odontiadis J, Murray BJ: Chromium treatment decreases the sensitivity of $5HT_{2A}$ receptors. Psychopharmacology (Berl), 2002, 159, 432–436.
4. Bleakman D, Alt A, Witkin JM: AMPA receptors in the therapeutic management of depression. CNS Neurol Disord Drug Targets, 2007, 6, 117–126.
5. Celada P, Puig M, Amargos-Bosch M, Adell A, Artigas F: The therapeutic role of 5-HT_{1A} and 5-HT_{2A} receptors in depression. J Psychiatry Neurosci, 2004, 29, 252–265.
6. Cryan JF, Leonard BE: 5-HT_{1A} and beyond: the role of serotonin and its receptors in depression and the antidepressant response. Hum Psychopharmacol, 2000, 15, 113–135.
7. Davidson JR, Abraham K, Connor KM, McLeod MN: effectiveness of chromium in atypical depression: a placebo-controlled trial. Biol Psychiatry, 2003, 53, 261–264.
8. Deakin JF: 5-HT_2 receptors, depression and anxiety. Pharmacol Biochem Behav, 1988, 29, 819–820.
9. Detke MJ, Rickels M, Lucki I: Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology, 1995, 121, 66–72.
10. Docherty JP, Sack DA, Hoffman M, Finch M, Komorowski JR: A double-blind, placebo-controlled, exploratory trial of chromium picolinate in atypical depression:

effect on carbohydrate craving. J Psychiatr Pract, 2005, 11, 302–314.
11. Dybała M, Siwek A, Poleszak E, Pilc A, Nowak G: Lack of NMDA-AMPA interaction in antidepressant-like effect of CGP 37849, an antagonist of NMDA receptor, in the forced swim test. J Neural Transm, 2008, 115, 1519–1520.
12. Franklin M, Odontiadis J: Effects of treatment with chromium picolinate on peripheral amino acid availability and brain monoamine function in the rat. Pharmacopsychiatry, 2003, 36, 176–180.
13. Hummel M, Standl E, Schnell O: Chromium in metabolic and cardiovascular disease. Horm Metab Res, 2007, 39, 743–751.
14. International Agency for Research on Cancer, World Health Organization, Chromium, Nickel and Welding. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, IARC, WHO, Lyon, Vol 49, 1999.
15. Katon W, Fan MY, Unützer J, Taylor J, Pincus H, Schoenbaum M: Depression and diabetes: A potential lethal combination. J Gen Intern Med, 2008, 23, 1571–1575.
16. Khanam R, Pillai KK: Effect of chromium picolinate on modified forced swimming test in diabetic rats: involvement of serotonergic pathways and potassium channels. Basic Clin Pharmacol Toxicol, 2006, 98, 155–159.
17. Leonard EE: Effects of depression on work loss and disability bed days in individuals with diabetes. Diabetes Care, 2004, 27, 1751–1753.
18. Maeng S, Zarate CA Jr, Du J, Schloesser RJ, McCammon J, Chen G, Maji HK: Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol Psychiatry, 2008, 63, 349–352.
19. McCarty MF: Enhancing central and peripheral insulin activity as a strategy for the treatment of endogenous depression: an adjuvant role for chromium picolinate? Med Hypotheses, 1994, 43, 247–252.
20. McLeod MN, Gaynes BN, Golden RN: Chromium potentiation of antidepressant pharmacotherapy for dysthymic disorder in 5 patients. J Clin Psychiatry, 1999, 60, 237–240.
21. McLeod MN, Golden RN: Chromium treatment of depression. Int J Neuropsychopharmacol, 2000, 3, 311–314.
22. NTP, 2000, National Toxicology Program, 9th edn, Report on Carcinogens, US Department of Health and Human Services, 2000.
23. Pilc A, Chaki S, Nowak G, Witkin JM: Mood disorders: regulation by metabotropic glutamate receptors. Biochem Pharmacol, 2008, 75, 997–1006.
24. Pittenger C, Sanacora G, Krystal JH: The NMDA receptor as a therapeutic target in major depressive disorder. CNS Neurol Disord Drug Targets, 2007, 6, 101–115.
25. Poleszak E, Szewczyk B, Wlaź A, Fidecka S, Wlaź P, Pilc A, Nowak G: D-serine, a selective glycine/NMDA receptor agonist, antagonizes the antidepressant-like effects of magnesium and zinc in mice. Pharmacol Rep, 2008, 60, 996–1000.
26. Poleszak E, Wlaź P, Kędzierska E, Nieoczym D, Wróbel A, Fidecka S, Pilc A, Nowak G: NMDA/Glutamate mechanism of antidepressant-like action of magnesium in forced swim test in mice. Pharmacol Biochem Behav, 2007, 88, 158–164.
27. Poleszak E, Wlaź P, Wróbel A, Dybała M, Sowa M, Fidecka S, Pilc A, Nowak G: Activation of the NMDA/glutamate receptor complex antagonizes the NMDA antagonist-induced antidepressant-like effects in forced swim test. Pharmacol Rep, 2007, 59, 595–600.
28. Porsolt RD, Bertin A, Jalfre M: Behavioural despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn, 1977, 229, 327–336.
29. Redrobe JP, Bourin M: Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the forced swimming test. Eur J Pharmacol, 1997, 325, 129–135.
30. Skolnick P: AMPA receptors: a target for novel antidepressants? Biol Psychiatry, 2008, 63, 347–348.
31. Skolnick P: Antidepressants for the new millennium. Eur J Pharmacol, 1999, 375, 31–40.
32. Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R: Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. Pharmacopsychiatry, 1996, 29, 23–26.
33. Skolnick P, Legutko B, Li X, Bymaster FP: Current perspectives on the development of non-biogenic amine-based antidepressants. Pharmacol Res, 2001, 43, 411–423.
34. Szewczyk B, Poleszak E, Sowa-Kućma M, Siwek M, Dudek D, Ryszewska-Pokraśniewicz B, Radziwoń-Zaleska M et al.: Antidepressant activity of zinc and magnesium in view of the current hypotheses of antidepressant action. Pharmacol Rep, 2008, 60, 588–599.

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