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INVOLVEMENT OF THE MONOAMINERGIC SYSTEM IN THE ANTIDEPRESSANT-LIKE ACTIVITY OF CHROMIUM CHLORIDE IN THE FORCED SWIM TEST

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Bio-metal chromium(III) is a crucial microelement for the proper functioning of living organisms. Previous preclinical and clinical studies reported its potential antidepressant properties. The aim of the present study was to examine the effect of antidepressants and noradrenergic and dopaminergic receptor antagonists on chromium chloride (CrCl_3) activity in the forced swim test (FST) in mice and rats. Imipramine (5 mg/kg), fluoxetine (5 mg/kg) and reboxetine (5 mg/kg) but not bupropion (1 mg/kg), administered jointly with CrCl_3 at a dose of 6 mg/kg, reduced the immobility time in the FST in mice. The reduction of the immobility time induced by the active dose (12 mg/kg) of CrCl_3 was completely abolished by propranolol (2 mg/kg, β -adrenoceptor antagonist), SCH 23390 (0.5 mg/kg, a dopamine D₁ receptor antagonist), and partially by prazosin (1 mg/kg, an α_1 -adrenoceptor antagonist), yohimbine (1 mg/kg, an α_2 -adrenoceptor antagonist) and sulpiride (50 mg/kg, a dopamine D₂/D₃ receptor antagonist) administration. The locomotor activity was significantly reduced by CrCl_3 + reboxetine treatment, which did not influence the reboxetine enhancement of the antidepressant-like effect of CrCl_3 in the FST. Moreover, CrCl_3 at a dose of 32 mg/kg (although not at 12 mg/kg) significantly reduced the immobility and enhanced the climbing (but not swimming) time in the FST in rats, which indicates the involvement of the noradrenergic pathway in this effect. The present study indicates that the antidepressant-like activity of chromium in the FST is dependent (although to a different extent) on the noradrenergic, dopaminergic and serotonin systems.

Key words: *antidepressants, adrenergic receptors, chromium chloride, dopaminergic receptors, forced swim test, locomotor activity, adrenergic antagonist*

INTRODUCTION

Chromium (Cr) can occur in nine oxidation states (from Cr(-II) to Cr(VI)). From all of these only Cr(III) is crucial for the function of living organisms, whereas Cr(VI) is highly toxic (1, 2, 3). It has been established that the metabolism of carbohydrates and lipids is contingent on the presence of Cr(III), while on the other hand its deficiency contributes to the pathophysiological mechanisms of diabetes (1). Cr(III) supplementation can improve insulin sensitivity (glucose tolerance) in diabetes, as demonstrated in experimental animals and patients (1, 4, 5, 6).

There is high co-morbidity between diabetes and depression (diabetic patients having two times more risk of depression than non-diabetics) which may increase the mortality threat (7, 8). The antidepressant activity of chromium(III) salts was demonstrated in both clinical and preclinical studies (9-13). The preclinical investigations indicated the involvement of the serotonin and glutamate systems in the chromium(III) antidepressant-like action (14, 15). Monoamine hypotheses of depression and antidepressant action were introduced almost 50 years ago (16-18), directly due to the role of noradrenaline, serotonin and dopamine systems in these mechanisms (19).

The aim of the present study was to examine the effect of antidepressant drugs with a different monoaminergic selectivity of the pharmacological mechanisms (imipramine, fluoxetine, reboxetine and bupropion), as well as noradrenergic (propranolol, prazosin, yohimbine) and dopaminergic (SCH 23390, sulpiride) receptor antagonists on CrCl_3 activity in the forced swim test (FST) in mice. Additionally, the involvement of the serotonergic/noradrenergic profile of action of CrCl_3 in the FST in rats was examined.

MATERIALS AND METHODS

Animals

All procedures were conducted according to the National Institutes of Health Animal Care and Use Committee guidelines and were approved by the Ethical Committee of the Jagiellonian University Medical College in Cracow. The experiments were carried out on male Albino Swiss mice (25–30 g) or male Wistar rats (230–250 g). The animals were housed under conditions with a constant temperature (20–22°C), a controlled 12:12 light-dark

cycle and free access to food and water. The experiments were carried out between 9:00 a.m. and 2:00 p.m.

Drug administration

Chromium chloride (CrCl_3 , Sigma-Aldrich, USA) and imipramine (Sigma-Aldrich, Germany) were administered 45 min before the test. Fluoxetine (Ascent, UK), reboxetine (Tocris, USA) and bupropion (Tocris, USA) were administered 30 mins before the test. Prazosin (Ascent, UK), yohimbine (Sigma-Aldrich, Germany), propranolol (Sigma-Aldrich, Germany), SCH 23390 (Tocris, USA) and sulpiride (Tocris, USA) were administered 60 mins before the test. The control animals received the vehicle (0.9% NaCl). All agents were administered intraperitoneally (i.p.).

Forced swim test

The studies were carried out on mice and rats according to the method of Porsolt and co-workers (20, 21).

The mice were propped individually into 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 mins. After the first 2 mins, the total duration of immobility was measured during a 4-mins test. The mouse was judged to be immobile when it remained floating passively in the water (20).

The rats were placed in glass cylinders (height 40 cm, diameter 20 cm) containing 30 cm of water, maintained at 25°C (21). Behavioral scoring was performed according to Detke *et al.* (22) and during the 5 min test session three different behaviors were rated: 1) immobility - where the rats were judged to be immobile when they remained floating passively in the water; 2) swimming - where the rats were judged to be swimming if they were making active swimming motions, additional to those solely used to maintain their heads above water; 3) climbing - where the rats were judged to be climbing when they were making active movements in and out of the water with their forepaws, usually directed against the walls.

Locomotor activity

The locomotor activity of the mice was measured with a photoresistor activity box (circular cages, diameter 25 cm, two

light beams). The animals were placed individually in an actometer for 2 mins. Activity was then measured for 4 mins. The number of crossings of the light beams by the mice was then recorded as the locomotor activity.

The locomotor activity of rats was measured in the elevated plus maze apparatus (an automated device produced by Kinder Scientific). Each rat was placed in the center of the plus maze, facing one of the closed arms. The number of ambulations during a 5-min test period (sum of walking movements in open and closed arms) was used as a marker of the locomotor activity.

Statistics

The data obtained was evaluated by the one-way or two-way analysis of variance (ANOVA), followed by Bonferroni's Multiple Comparison Test. All the results are presented as the means \pm S.E.M.; with $p < 0.05$ being considered as statistically significant.

RESULTS

Effect of combined administration of CrCl_3 and imipramine in the FST (Fig. 1)

Imipramine (5 mg/kg) or CrCl_3 (6 mg/kg), administered by themselves, had no effect on the immobility time, but when they were administered together they significantly reduced the immobility time. The two-way ANOVA revealed a significant effect of imipramine $F(1,31)=39.60$, $p=0.0001$, no effect of CrCl_3 $F(1,31)=3.20$, $p=0.0836$ and significant interaction $F(1,31)=7.27$, $p=0.0113$.

Effect of combined administration of CrCl_3 and fluoxetine or reboxetine in the FST (Fig. 2)

CrCl_3 (6 mg/kg), administered by itself, had no effect on the immobility time, but when administered together with fluoxetine (5 mg/kg) or reboxetine (5 mg/kg) it significantly reduced the immobility time. Imipramine (30 mg/kg), administered as a positive control, induced a significant reduction in the immobility time. The one-way ANOVA $F(5,50)=19.41$, $p=0.0001$.

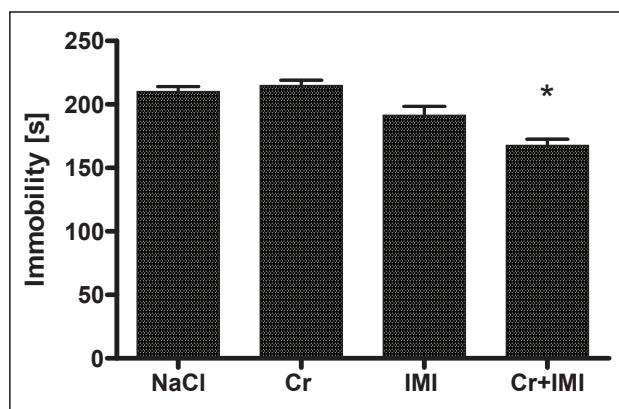


Fig. 1. The effect of co-treatment with a subactive dose of CrCl_3 (Cr, 6.0 mg/kg) combined with a subactive dose of imipramine (IMI, 5.0 mg/kg) on the total duration of immobility in the FST in mice. The values represent the mean \pm S.E.M. (n= 9–12). * $p < 0.001$ vs. control (NaCl), $p < 0.001$ vs. CrCl₃ group (Cr), $p < 0.05$ vs. imipramine treated group (IMI). (Bonferroni's Multiple Comparison Test).

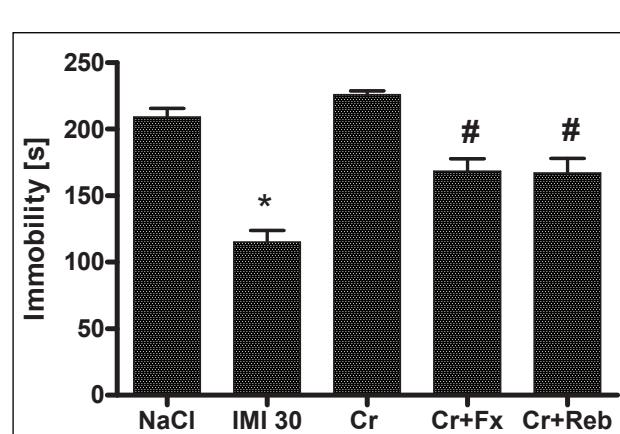


Fig. 2. The effect of co-treatment with a subactive dose of CrCl_3 (Cr, 6.0 mg/kg) combined with subactive doses of fluoxetine (Fx, 5 mg/kg) or reboxetine (Reb, 5 mg/kg) on the total duration of immobility in the FST in mice. The values represent the mean \pm S.E.M. (n= 6–12). * $p < 0.001$ vs. control (NaCl), # $p < 0.01$ vs. CrCl₃ group (Bonferroni's Multiple Comparison Test).

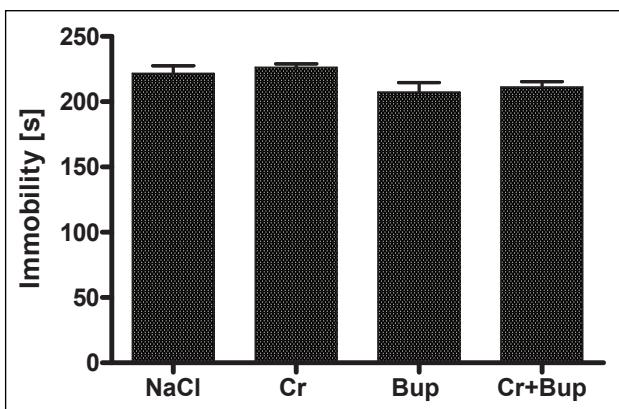


Fig. 3. The effect of co-treatment with a subactive dose of CrCl_3 (Cr, 6.0 mg/kg) combined with a subactive dose of bupropion (Bup, 1 mg/kg) on the total duration of immobility in the FST in mice. The values represent the mean \pm S.E.M. ($n=6-10$).

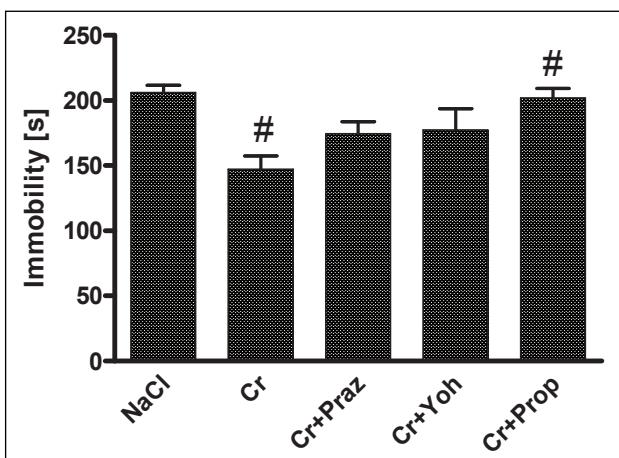


Fig. 4. The effect of the CrCl_3 (Cr) treatment in combination with prazosin (Praz), yohimbine (Yoh) or propranolol (Prop), on the total duration of immobility in the FST in mice. The values represent the mean \pm S.E.M. ($n=8-10$). # $p<0.01$ vs. control (NaCl), # $p<0.01$ vs. CrCl_3 group (Cr) (Bonferroni's Multiple Comparison Test).

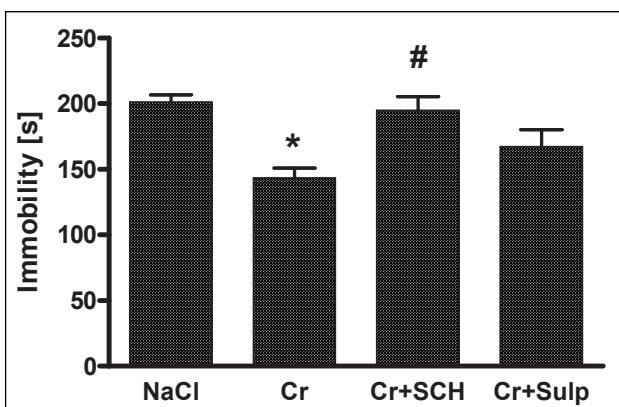


Fig. 5. The effect of the CrCl_3 (Cr) treatment in combination with SCH 23390 (SCH) or sulpiride (Sulp) on the total duration of immobility in the FST in mice. The values represent the mean \pm S.E.M. ($n=10-12$). * $p<0.001$ vs. control (NaCl), # $p<0.01$ vs. CrCl_3 group (Cr) (Bonferroni's Multiple Comparison Test).

Effect of combined administration of CrCl_3 and bupropion in the FST (Fig. 3)

CrCl_3 (6 mg/kg) or bupropion (1 mg/kg), administered by themselves or together, had no effect on the immobility time. The two-way ANOVA revealed no effect of bupropion $F(1,27)=7.58$, $p=0.0104$, no effect of CrCl_3 $F(1,27)=0.03$, $p=0.8667$ and no interaction $F(1,27)=0.29$, $p=0.5920$.

The effect of the combined administration of CrCl_3 and adrenergic antagonists in the FST (Fig. 4)

CrCl_3 (12 mg/kg), administered by itself, significantly reduced the immobility time. Prazosin (1 mg/kg) or yohimbine (1 mg/kg), administered together with CrCl_3 , did not significantly alter the immobility time. The CrCl_3 -induced reduction of the immobility time was significantly counteracted by propranolol (2 mg/kg). The one-way ANOVA $F(4,40)=5.118$, $p=0.002$.

The effect of the combined administration of CrCl_3 and dopaminergic antagonists in the FST (Fig. 5)

CrCl_3 (12 mg/kg), administered by itself, significantly reduced the immobility time. Sulpiride (50 mg/kg) administered together with CrCl_3 , did not significantly alter the immobility time. The CrCl_3 -induced reduction of the immobility time was significantly counteracted by SCH 23390 (0.5 mg/kg). The one-way ANOVA $F(3,38)=8.333$, $p=0.0002$.

The effect of the combined administration of CrCl_3 and antidepressants or noradrenergic or dopaminergic receptor antagonists on locomotor activity in mice (Table 1)

CrCl_3 , combined with prazosin, yohimbine, propranolol, SCH 23390, sulpiride, imipramine, fluoxetine or bupropion, had no effects on the locomotor activity in mice. However, CrCl_3

Table 1. Effect of chromium chloride (Cr) combined with adrenergic (A), dopaminergic (B) ligands and co-treatment with antidepressants (C) on locomotor activity in mice. CrCl_3 (Cr) was administered i.p. 45 min before the test. Prazosin (Praz), yohimbine (Yoh), propranolol (Prop), SCH 23390 (SCH) and sulpiride (Sulp) was administrated 60 min before the test. Imipramine (IMI), fluoxetine (Fx) and reboxetine (Reb) were administered 0.5 hours before the test. The values represent mean \pm S.E.M. ($n=6$ mice per group). * $p<0.05$ vs. control (NaCl) (Bonferroni's Multiple Comparison Test).

Treatment	Dose (mg/kg)	Activity counts
A		
control		68.3 ± 14.7
Cr+Praz	12+1	41.7 ± 13.2
Cr+Yoh	12+1	60.2 ± 24.6
Cr+Prop	12+2	61.0 ± 21.3
B		
control		68.4 ± 16.4
Cr+SCH	12+0.5	30.3 ± 14.8
Cr+Sulp	12+50	44.3 ± 12.1
C		
control		89.8 ± 8.2
Cr+IMI	6+5	76.2 ± 21.0
Cr+Fx	6+5	76.2 ± 9.4
Cr+Reb	6+5	$58.0 \pm 9.4^*$
Cr+Bup	6+1	87.2 ± 14.4

plus reboxetine treatment significantly reduced the locomotor activity by 35% (*Table 1C*).

The effect of the administration of CrCl₃ on immobility, swimming and climbing time in the FST in rats (Fig. 6)

Imipramine (30 mg/kg) and CrCl₃, at a dose of 32 yet not 12 mg/kg, significantly reduced the immobility time (Fig. 6A; The one-way ANOVA $F(3,32)=5.641$, $p=0.0042$). None of the tested agents significantly altered the swimming time (Fig. 6B); The one-

Table 2. Effect of chromium chloride on locomotor activity in rats. CrCl₃ (Cr) was administered i.p. 60 min before the test. The values represent mean \pm S.E.M. (n=6 rats per group).

Treatment	Dose (mg/kg)	Activity counts
control		185.3 \pm 59.0
Cr	6	131.7 \pm 43.8
Cr	12	140.4 \pm 37.4
Cr	32	153.4 \pm 62.6

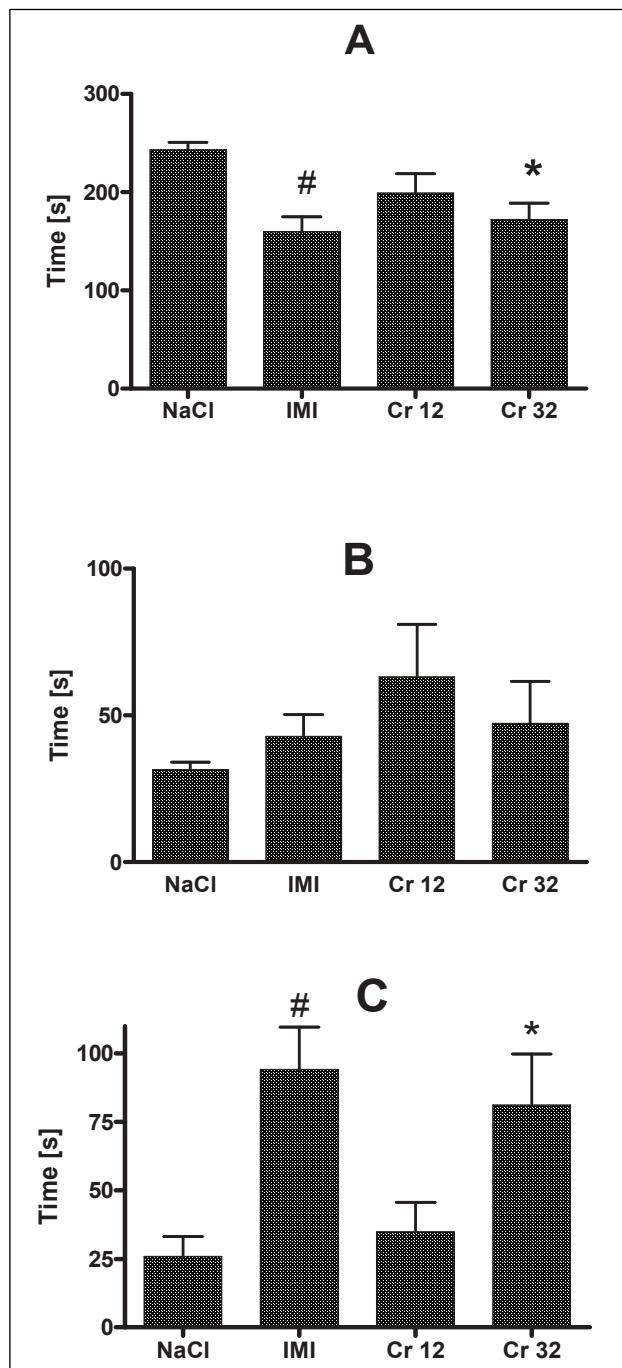


Fig. 6. The effect of the CrCl₃ (Cr) or imipramine (IMI) treatment on the total duration of immobility (A), swimming (B) and climbing (C) in the FST in rats. The values represent the mean \pm S.E.M. (n=8–10). # $p<0.01$ vs. control (NaCl), * $p<0.05$ vs. control (NaCl) (Dunnett test).

way ANOVA $F(3,32)=1.147$, $p=0.3449$). However, imipramine and CrCl₃ (32 mg/kg) significantly increased the climbing time (Fig. 6C; One-way ANOVA $F(3,32)=6.409$, $p=0.0016$).

The effect of CrCl₃ administration on the locomotor activity in rats (Table 2)

CrCl₃, administered at doses of 6, 12 or 32 mg/kg, had no effects on the locomotor activity in rats.

DISCUSSION

Chromium picolinate salt, administered 400–600 μ g/day, was effective in patients suffering from a major depressive disorder (studies performed as the single- or double-blind clinical trials; (9, 10, 13), reviewed by Iovieno *et al.*, (23)). Moreover, the beneficial effect of the chromium supplementation to pharmacotherapy was also reported in endogenous or dysthymic affective disorders (11, 12). These clinical studies suggest the possible antidepressant properties of chromium in affective disorders (13).

The present data generally confirmed the antidepressant-like activity of chromium and pointed to the involvement of the monoamine system in this activity. The previous studies by Khanam and Pillai (15) indicated the participation of the serotonin system in the antidepressant-like effect of chromium picolinate in the modified rat FST (22). This assumption was based on their results that chromium (when used chronically), besides reducing the immobility time, increased the swimming yet not climbing parameter of this test (15). Our present data are different, and are indicating that the noradrenergic pathway is connected with the chromium effect in the FST in rats, since the climbing yet not the swimming is increased. This discrepancy may be due to different rat strains and/or different ways, schedules, doses and salts of chromium treatment. All the data suggest that both serotonergic and noradrenergic pathways are involved in the antidepressant-like action of chromium in the FST in rats. Our previous study (24), using the mouse FST demonstrated that the antidepressant-like activity of chromium chloride is partially dependent on 5-HT_{1A} and the 5-HT_{2A} serotonin receptor. This serotonin mechanism was also supported by the present data that antidepressants using the serotonin mechanism of pharmacology (imipramine, fluoxetine) potentiate the antidepressant activity of chromium in the FST in mice.

On the other hand, the noradrenergic mechanism was indicated by the following recent results: antagonism of chromium activity by adrenergic receptor antagonists (propranolol, prazosin, yohimbine) and potentiation of the chromium antidepressant-like action by reboxetine (the selective noradrenaline uptake inhibitor), in spite of the reduction of the locomotor activity demonstrated by such a drug combination. Moreover, the contribution of the dopamine

system was also designated, since dopamine D-1(SCH 23390) and D-2/3(sulpiryd) receptor antagonists partially or completely antagonized the antidepressant-like action of chromium chloride in the FST.

The involvement of the monoamine system in the biological/pharmacological activity of chromium was supported by human and animal studies of Attenburrow *et al.* (14), and Franklin and Odontiadis (25), who demonstrated an increase of the serotonin level and metabolism, and "down-regulation" of the serotonin 5HT_{2A} receptors, following chromium picolinate administration.

Other monoaminergic mechanisms of chromium antidepressant activity were also proposed. Our previous study indicated the glutamatergic system (AMPA and NMDA receptors) as being involved in the antidepressant-like action of chromium in the FST in mice (24). Furthermore, Khanam and Pillai (15) suggested the involvement of the K⁺ channel in the antidepressant activity of chromium, because glimeperide (a K⁺ channel antagonist) potentiates the chromium effect in the swimming time. However, since glimeperide did not affect the immobility time of the chromium activity (the core parameter of the FST), this data questions the relation of that channel to chromium antidepressant-like action (11, 21, 22).

As mentioned in the Introduction, the monoamine theory of depression was proposed 50 years ago (16-18), and with current modifications still receiving attention and being investigated (19, 26-28), however, other systems are also under continuous examinations, e.g. opioids (29). Thus, the antidepressant monoaminergic mechanism of chromium is worth being further examined.

In summary, the present study indicates that the antidepressant-like activity of chromium in the FST is dependent (to a different extent) on the noradrenergic, dopaminergic and serotonin systems.

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Conflict of interest: None declared.

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