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Vitamin C as Potential Alternative Treatment For Depression And Anxiety

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Abstract

Mood disorders increase sickness, death, and costs. Ascorbic acid (AA), a water-soluble vitamin, can help cure depression. Unknown anti-manic properties. The current study used the tail suspension test (TST) to evaluate the opioid system's role in AA 's antidepressant-like effects. This study examined if AA could assist m-Amphetamine users relax. AA administered into the TST did not lessen the time Swiss mice remained immobile after receiving naloxone. Naloxonazine, a selective opioid receptor 1-type antagonist, decreased the antidepressant-like effects of AA on the TST while not affect mobility (OFT). When naloxonazine (NLX) and alcohol were given to rats' parahippocampal and prefrontal cortex, PSD95 levels did not alter. Wistar rats were given distilled water, lithium chloride (LiCl), or AA for 14 days. Starting on day 7, m-Amphetamine or the vehicle was injected. The mice were given a single dose of mAMPH and tested two hours later. After being fed m-Amphetamine, animals roamed and explored more. AA can't prevent this behaviour, but lithium chloride can. m-main AMPH lowers the immunocontent of B.D.N.F in the rat parahippocampal region. In the m-Amphetamine group, 10 mg/kg AA inhibited B.D.N.F production. m-Amphetamine increased FGF-2 levels in the prefrontal cortex, but FGF-2 immunocontent did not change across groups. First, AA 's antidepressant-like effect on TST appears to depend on opioid system activation, namely 1-type receptor activation. Second, stimulation of the mTOR- PSD95 pathway, which was involved in AA 's antidepressant-like effect, seems unrelated to AA 's opioid system activation. AA had no effect on the m-Amphetamine-induced mania animal model, and B.D.N.F and FGF-2

may be involved in its manifestation. This information may help determine AA 's involvement in mood modulation.

Keywords: Ascorbic acid, mania, m-Amphetamine, B.D.N.F, FGF-2, depression, the opioid system, receptors, and PDS95

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خلاصة

اضطرابات المزاج تزيد من المرض والموت والتكاليف. يمكن أن يساعد حمض الأسكوربيك (AA)، وهو فيتامين قابل للذوبان في الماء، في علاج الاكتئاب. خصائص مضادة للهوس غير معروفة. استخدمت الدراسة الحالية اختبار تعليق الذيل (TST) لتقييم دور نظام المواد الأفيونية في التأثيرات الشبيهة بمضادات الاكتئاب لـ AA. بحثت هذه الدراسة فيما إذا كان AA يمكن أن يساعد مستخدمي m-Amphetamine على الاسترخاء. لم يقلل AA المعطى في اختبار TST من الوقت الذي ظلت فيه الفئران السويسرية غير متحركة بعد تلقي النالوكسون. نالوكسونازين، وهو مضاد انتقائي لمستقبلات المواد الأفيونية من النوع 1، قلل من التأثيرات الشبيهة بمضادات الاكتئاب لـ AA على TST بينما لا يؤثر على الحركة (OFT). عندما تم إعطاء النالوكسونازين (NLX) والكحول للقشرة المجاورة للحصين وقشرة الفص الجبهي لدى الفئران، لم تتغير مستويات PSD95. تم إعطاء فئران ويستار الماء المقطر أو كلوريد الليثيوم (LiCl) أو AA لمدة 14 يومًا. ابتداءً من اليوم السابع، تم حقن الأمفيتامين أو المركبة. أعطيت الفئران جرعة واحدة من mAMPH وتم اختبارها بعد ساعتين. بعد إطعامها الأمفيتامين، تجولت الحيوانات واستكشفت المزيد. لا يستطيع AA منع هذا السلوك، لكن كلوريد الليثيوم يمكنه ذلك m-main AMPH. يخفض المحتوى المناعي لـ B.D.N.F في منطقة الفئران المجاورة للحصين. في مجموعة m-Amphetamine ، أدى 10 ملغم/كغم من AA إلى تثبيط إنتاج B.D.N.F. زاد الأمفيتامين من مستويات FGF-2 في قشرة الفص الجبهي، لكن المحتوى المناعي لـ FGF-2 لم يتغير عبر المجموعات. أولاً، يبدو أن تأثير AA المضاد للاكتئاب على TST يعتمد على تنشيط نظام المواد الأفيونية، أي تنشيط مستقبل من النوع الأول. ثانيًا، يبدو أن تحفيز مسار mTOR-PSD95 ، والذي كان مشاركًا في التأثير الشبيه بمضاد الاكتئاب لـ AA ، لا علاقة له بتنشيط نظام المواد الأفيونية لـ AA. لم يكن لـ AA أي تأثير على النموذج الحيواني للهوس الناجم عن الأمفيتامين، وقد يكون

B.D.N.Fو FGF-2متورطين في ظهوره. قد تساعد هذه المعلومات في تحديد مدى مشاركة AA في تعديل الحالة المزاجية.

الكلمات المفتاحية: حمض الأسكوربيك، الهوس، الأمفيتامين، B.D.N.F، FGF-2، الاكتئاب، نظام المواد الأفيونية، المستقبلات، وPDS95

Introduction

Depression is one of the most common neuropsychiatric conditions in the Western world, affecting roughly 280 million individuals and having a lifetime prevalence of approximately 21% in the population. The American Psychiatric Association's criteria for diagnosing severe depression are based on clinical observation of symptoms (Luqman, 2024). It is critical to underline that symptoms must be present for at least two weeks and at least one of them must be sad mood or anhedonia most of the time. Some investigations have consistently found a link between oxidative stress and significant depression. The production of free radicals in aerobic organisms is a constant and physiological process that results from metabolic processes such as enzyme activity, electron transport mechanisms, and the oxidation of soluble substances in the cytosol. Its creation, in the proper quantities, allows for adenosine triphosphate (ATP) production via the electron transport chain. Excessive generation of free radicals, on the other hand, might cause oxidative damage. (Xu, 2018) When there is an imbalance between oxidant and antioxidant chemicals, either in favour of the excessive creation of free radicals or to the disadvantage of their removal speed, the oxidative stress process is activated. This process oxidises biomolecules such as lipids, proteins, and DNA, causing damage to their biological activities and homeostatic imbalance. Modern antidepressant therapy comprises psychological and physical interventions and antidepressant medicines. Supportive psychotherapy, interpersonal, behavioural, cognitive behavioural, group, couple, and family therapy are all examples of psychotherapeutic therapies. Only in moderate depression are such treatments helpful as a standalone treatment; otherwise, they are used in conjunction with antidepressants. (Vandeleur, 2017)

Antidepressants are classified into numerous classes, including tricyclics, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, and combined serotonin and norepinephrine reuptake inhibitors. However, these medications only give complete remission for roughly 50% of patients, with many (more than 80%) exhibiting partial responses and causing numerous side effects. Furthermore, the therapeutic response to these medications is not quick, typically occurring between the second and fifth weeks after treatment begins. (Baxter, 2016) Given the low clinical efficacy and numerous side effects caused by the drugs used to treat depression, which frequently contribute to the patient discontinuing treatment, there is a great need for the development of alternative antidepressant therapies or substances that can optimise the clinical efficacy of

depression treatment. (Nakimuli-Mpungu, 2011) Concerns about the creation of novel antidepressant medications with mechanisms different from the current ones, that are more effective and act faster, has led to research into the etiological grounds of depression. In this setting, animal models of depression are critical for understanding and progressing research on this condition.

Furthermore, there have been indications that Ascorbic acid (AA) has an antidepressant impact in preclinical and clinical research, therefore it is essential better to understand the molecular mechanisms of action of this vitamin. On the other hand, in addition to the antidepressant effect of Ascorbic acid, there is evidence that this vitamin may have antimanic properties (Carr, 2013), making the investigation of this compound's action in an animal model of mania interesting. In this method, we could learn more about the direct and indirect molecular targets implicated in Ascorbic acid's mood-regulating impact. Given the foregoing, this study hypothesises: a) that AA exerts an antidepressant action via opioid system modulation, which could indicate a promising effect in cases of refractory depression; and b) that AA exerts an antimanic action, and may become an alternative to current pharmacological treatments.

Animal models (Goyal, 2016) of depression with phenomenology and conceptual validity are employed in addition to predictive validity. Models based on stress induction and pharmaceutical models for depression stand out among these. Stress induction models are based on epidemiological studies that link stressful life experiences as the primary environmental risk factor for depression onset. Animals in these models are subjected to numerous stressors and, as a result, exhibit depressive-like behaviours. Furthermore, the importance of animal models of stress-induced depression is demonstrated by the fact that therapy with antidepressants reverses abnormalities in behavioural measures. There are two types of stress-induced animal models of depression: mild chronic stress and unpredictable stress.

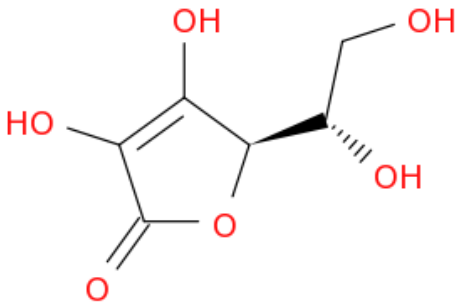


Figure 1: L-acid ascorbic or Vitamin-C

L-acid ascorbic ($C_6H_8O_6$-Figure 1), often known as vitamin C, is an antioxidant water-soluble vitamin that engages in a variety of critical tasks and has the characteristic of power reduction. AA performs a variety of critical tasks related to its oxidation-reduction capacity. It has A well-documented enzymatic

cofactor in hydroxylation processes, such as the hydroxylation catalysed by the enzyme's prolyl and lysyl hydroxylase. (Moretti, 2013) In these reactions, hydroxyl groups are added to the waste of proline and lysine in collagen molecules, which helps to increase the stability of the molecule's triple helix structure. A function for ascorbic acid in mood disorders has been suggested by clinical findings of recovery from idiopathic (unknown aetiology) depression with high doses of ascorbic acid, as well as depression associated with ACTH use in one child with hepatitis. One study also found that giving ascorbic acid to healthy people for 14 days reduced their Beck depression inventory ratings. Another study discovered a drop in ascorbic acid levels in the plasma of people suffering from severe depression. Furthermore, a clinical investigation described O case in a depressed patient who got scurvy (Brody, et al., 2002), which implies that one ingestion inadequate of this Vitamin, and consequently low endogenous levels of ascorbate, can be connected. Depression is caused by the pathophysiology. Furthermore, additional research has found one comorbidity between scurvy and depression. In animals, rats treated to continuous mild stress had one drop in ascorbic acid level in the cerebral cortex. Furthermore, our research discovered that ascorbic acid has an antidepressant impact on TST in mice, including activating monoaminergic systems. The current study sought to assess the behavioural and biochemical effects of ascorbic acid administration in animal models of depression and the potential pathways involved in this vitamin C's antidepressant-like impact.

2. MATERIAL AND METHODS

2.1 ANIMALS

Female Swiss mice were about 50 days old and weighed between 30 and 40g were used to study the role of the opioid system in how ascorbic acid acts like an antidepressant. The animals were kept in permanent cages that were 41 cm by 34 cm by 16 cm. There were 15 animals in each box, and they were put into one of eight experimental groups at random. Male Wistar rats that were 2 months old and weighed between 250 and 300 grammes were used to study the effect of ascorbic acid on m-Amphetamine-induced mania. The animals lived in groups of five in cages 41 cm by 34 cm by 16 cm. For the experiments, the animals were put into groups of 6 to 12 by chance. In both sets of experiments, the animals were kept in a room with a temperature of 22±1ºC. They had free access to food and water and were on a 12:12h light/dark cycle.

2.2 DRUGS

Naloxone (a non-selective antagonist of opioid receptors) and NLX (an antagonist of □-opioid receptors) were used to examine the antidepressant effect of AA (Merck-India) (SD Fine chemicals, India). AA (10 ml/kg) dissolved in sterile water was given orally. All solutions for the intraperitoneal

administration of naloxone and naloxonazine (10 ml/kg) were produced just prior to treatment and diluted with saline (NaCl 0.9%). AA (0.2, 0.5, and 50mg/kg, Merck-India), lithium chloride (Merck-India), and m-amphetamine were utilised to test Ascorbic acid's mood-stabilizing effects (Merck-India). In a total amount of 1 ml/kg, m-Amphetamine, Lithium chloride, and ascorbic acid were injected intraperitoneally. The oral and intraperitoneal solutions, made from scratch each day of therapy, were distilled water for compounds taken by mouth and saline solution (NaCl 0.9% for compounds taken by injection) (Binfaré, 2009).

2.3 INVESTIGATION OF THE ANTIDEPRESSANT ACTION MECHANISM OF ASCORBIC ACID

Animals were given AA (1 mg/kg, p.o), naloxone (1 mg/kg, i.p.) 30 minutes later, and then analysed for behaviour in the TST and OFT (open-field test) after 30 minutes to find out what role the opioid system plays in Ascorbic acid's antidepressant-like effect (Figure 2).

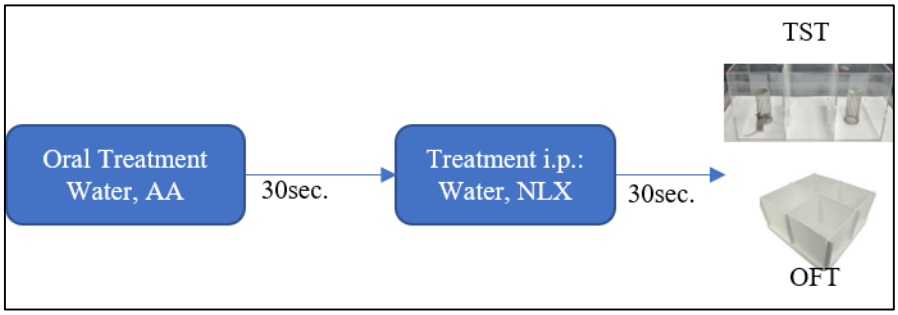


Figure 2: Experimental protocol-1.

The Experimental Procedure, Shown in Figure 3 In order to determine the effect of co-administering AA and the non-selective opioid receptor antagonist naloxone, the two drugs were studied in combination. After 30 minutes, the animals were evaluated on the TST and OFT after receiving an oral dose of ascorbic acid (1 mg/kg) and an intramuscular dose of naloxone (1 mg/kg).

Animals were given 2mg/kg of ascorbic acid and then 10mg/kg of NLX; 30 minutes later to study the particular involvement of 1-opioid receptors. Following a 30-minute resting period, the animals were subjected to the aforementioned behavioural assessments. After the experiments were complete, the animals in these groups had their heads removed, their parahippocampal regions and prefrontal cortices dissected on ice, and their brains were flash frozen in liquid nitrogen and preserved at -80°C for later biochemical examination (Figure 3).

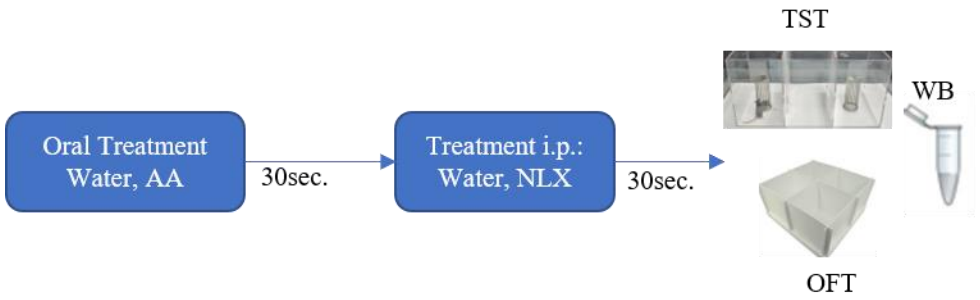


Figure 3: Experimental protocol-2.

The Experimental Procedure, as Depicted in Figure 3 AA and naloxone, a selective opioid antagonist for 1-type receptors, were tested in combination to see what would happen. Animals received an oral dose of ascorbic acid (1 mg/kg), then 30 minutes later, a dose of NLX (10 mg/kg), and finally, 30 minutes later, the animals were tested behaviorally and had their parahippocampal region and prefrontal cortex dissected and prepared for immunodetection of proteins via Western blotting (WB).

2.4 INVESTIGATION OF THE EFFECT OF AA IN AN ANIMAL MODEL OF MANIA

AA (0.2, 0.5, and 50 mg/kg), lithium chloride (50 mg/kg p.o.), and distilled water (1 ml/kg) were given to rats twice daily for 14 days in an m-Amphetamine-induced mania paradigm. Beginning on day 8, each of the 12 treatment groups received either m-Amphetamine (2 mg/kg) or saline (1 ml/kg) intraperitoneally once daily for 7 more days. Locomotor activity in the PFT was measured 2 hours after a single injection of m-Amphetamine or saline was given on the 15th day of therapy. Immediately following the experiment, the animals were sacrificed and their brains were physically dissected on ice before being snap-frozen in liquid nitrogen and preserved at -80°C for further biochemical examination (see Figure 4).

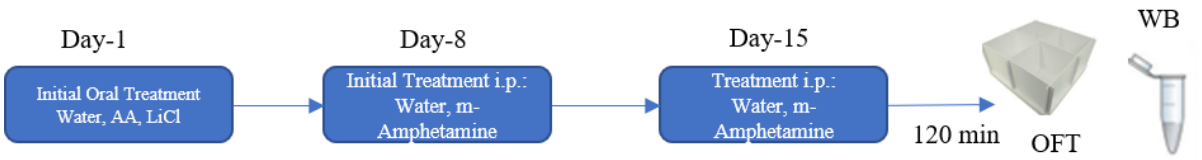


Figure 4: Experimental protocol-3

A third study studied the flowchart of the Experiment Ascorbic acid's impact on preventing m-Amphetamine-induced mania. For 14 days, the animals were given two doses of either ascorbic acid (0.2, 0.5, and 50mg/kg), lithium chloride (50 mg/kg), or distilled water (1 ml/kg) orally. Starting on day 8, patients received either m-Amphetamine (2mg/kg) or saline (1ml/kg) by injection once

daily for the next 7 days. Animals were given a single dose of m-Amphetamine on day 15 and then assessed on the PFT and their parahippocampal region and prefrontal cortex were dissected for Western Blot protein immunocontent analysis 2 hours later (WB).

2.5 BEHAVIORAL EVALUATIONS

2.5.1 TST: The TST is known as a predictive test for the antidepressant-like action of a compound, since classic antidepressants decrease the immobility time of animals in this test. During the test, the animals are acoustically and visually isolated and suspended 50 cm from the ground for 6 minutes, while the time they spend immobile is timed (Pemminati, et al.2010).

2.5.2 OFT: The OFT is a 40x60x50 cm wooden box with a foundation that is divided into 12 equal squares. An indicator of locomotor activity is the number of squares traversed by all four paws during a time period of either 5 minutes (in rats) or 6 minutes (in mice). Between each set of testing, the bottom of the wooden box was wiped down with 10% alcohol in a dark, temperature- and light-controlled environment.

AA has been shown to have antidepressant-like effects, hence OFT is utilised to rule out the hypothesis that increased animal locomotion caused the reduction in immobility time in TST in the associated investigation. Notably, conventional antidepressants shorten the inactivity phase of the TST without inducing motor changes in the PFT (Moretti et al., 2020).

A rat model using OFT is utilised to predict the effect of ascorbic acid on mood stabilisation. When animals are given an m-Amphetamine-induced mania model, their locomotor activity increases in the PFT. Mood-stabilizing medications, including lithium chloride, can reverse this increase in movement (Marik, 2018). The number of lifts, where the animal stands only on its hind legs and lifts its body vertically, is also counted in this protocol, and this is another metric that the model affects.

2.6 BIOCHEMICAL EVALUATIONS - IMMUNODETECTION OF PROTEINS BY WESTERN BLOT

In all procedures, parahippocampal and prefrontal proteins were immunodetected by Western blotting. Samples were prepared using Oliveira et al. (2008) procedures. Tissues were homogenised in 300 L of solution. After centrifuging lysates at 10,000 x g for 10 minutes at 4°C, the supernatant (200 L) was diluted 1/1 (v/v) in 100 mM Tris pH 6.8, 4mM EDTA, SDS 8% and heated at 100°C for 5 minutes to eliminate cellular debris. A homogenised aliquot was used to evaluate protein content. 8% -mercaptoethanol and 25% sample dilution solution were then added.

Protein content and concentration were estimated using Peterson's bovine serum albumin standard curve (Amos, 2015). Proteins (60 g total protein/well) were electrophoretically separated on a polyacrylamide gel containing SDS (SDS-PAGE) with 10% or 12% acrylamide concentrations, depending on the protein of interest, and an entry gel containing 4% acrylamide. Proteins were stored in nitrocellulose membranes. To confirm the transfer, membranes were stained with 0.5% Ponceau in 1% acetic acid.

Membranes were then occluded for an hour with skim milk powder (5% TBS) (10 mM Tris, 150 mM NaCl, pH 7.5). PSD95, FGF-2, and B.D.N.F were detected using 1:1000 dilutions of specific antibodies in TBS-T containing bovine serum albumin. Membranes were incubated with primary antibody overnight at 4°C with agitation. To detect immune complexes, membranes were incubated overnight with a peroxidase-conjugated anti-rabbit secondary antibody (1:5000). The next day, the immunoreactivity of the bands was revealed by chemiluminescence. After each blocking and incubation round, membranes were washed in TBS-T for five minutes. Anti-actin antibody (1:1000) confirmed equal protein loading on gel. Brain-Derived Neurotrophic Factor (B.D.N.F) and FGF-2 levels were detected in the same membrane by eliminating immunocomplexes (Matsuda, et al., 2012). After 5 minutes in 0.2 M NaOH, membranes were rinsed in doubly deionized water, washed twice, and incubated in TBS-T. (5 minutes). Immunocomplex-free membranes were blocked and treated similarly. The OD was calculated and presented as a percentage change from the baseline (100%).

2.7 STATISTICAL ANALYSIS

Two-way analysis of variance (ANOVA) and Duncan's post-hoc test (where applicable) were used to analyse the data acquired for both behavioural and biochemical markers. When the probability level was below <0.05, it was considered significant. Statistica 7.0 was utilised for all of the statistical analysis.

3. RESULTS

3.1 INVESTIGATION OF THE ANTIDEPRESSANT ACTION MECHANISM OF AA

3.1.1 Ascorbic acid's antidepressant effects in the TST and its effect on locomotor activity in the OFT both involve the opioid system.

In light of previous research suggesting that AA affects many neurotransmitter systems, the current study sought to determine whether or not it also affects the opioid system. The initial set of research aimed to do just that, and they made use of the non-selective opioid receptor antagonist naloxone. The result of naloxone's (1 mg/kg, i.p.) suppression of opioid receptors is depicted in Figure

5A. Using a two-way analysis of variance, we find that there are statistically significant differences between the AA treatment group and the naloxone treatment group and between the two treatment groups and their interaction group, the naloxone treatment group and its interaction group [and the two treatment groups. Post hoc analysis reveals that animals' TST immobility time was significantly shortened after receiving 1 mg/kg of AA by systemic injection ($P<0.01$), but was not shortened after receiving i.p. naloxone. As can be seen in Figure 5B, there were no statistically significant differences in the total number of crossings made by the animals during the PFT between any of the groups subjected to a locomotor activity analysis (treatment with Ascorbic acid, treatment with naloxone, interaction of the two treatments.

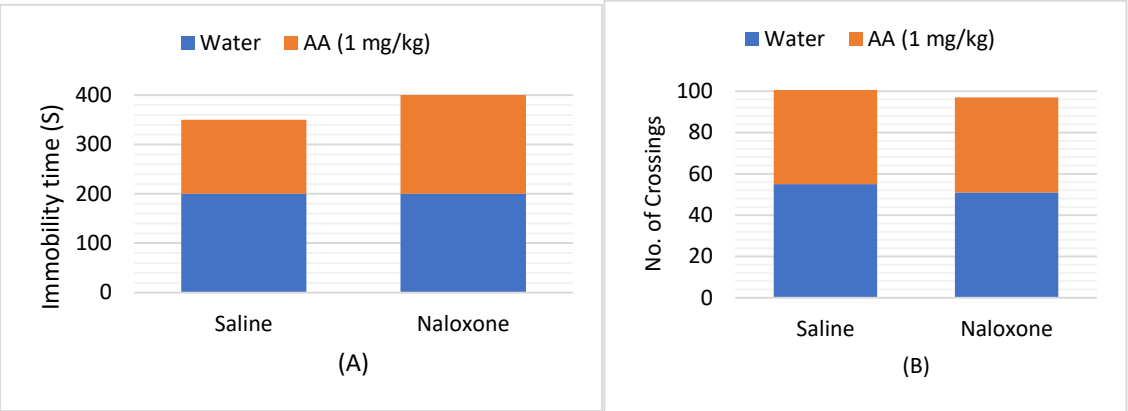


Figure 5. Effect of pre-treatment with naloxone (0.2 mg/kg, i.p.) on the anti-immobility effect of AA in TST (A) and on locomotion in OFT (B).

3.1.2 Involvement of μ1-type opioid receptors in the antidepressant-like effect of AA on TST and locomotor activity on OFT

Deepening our knowledge, a little, in another group of experiments, we used a selective opioid receptor antagonist for the μ1 type, NLX. Figure 6A shows the effect of inhibition of μ1 opioid receptors by NLX, two-way ANOVA revealed significant differences for treatment with NLX and for the interaction of AA treatment with NLX treatment, but not for AA treatment. The post-hoc analysis showed that the systemic administration (p.o.) of AA at a dose of 1 mg/kg significantly reduced the immobility time of the animals in the TST ($P<0.01$), while the i.p. of NLX was able to prevent this effect significantly. The analysis of locomotor activity did not show significant changes in the number of crossings of the animals in the PFT in any of the groups (treatment with Ascorbic acid, treatment with NLX, interaction of the two treatments (Figure 6B).

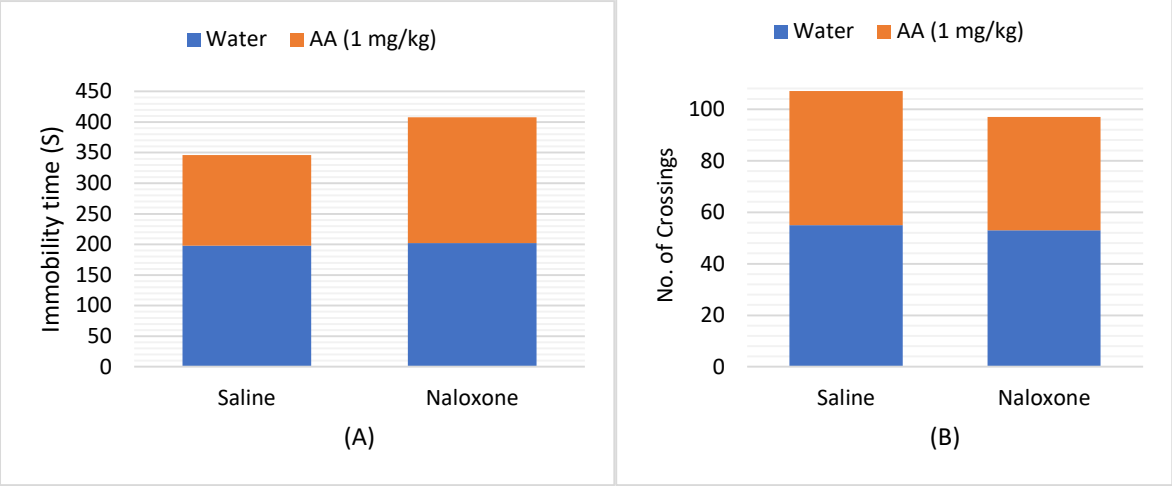
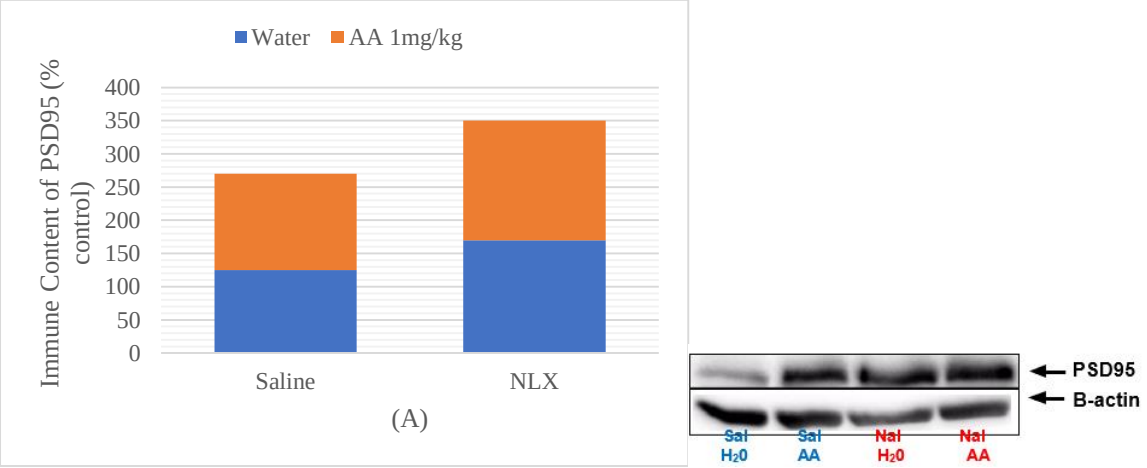


Figure 6: Effect of pre-treatment of animals with NLX (0.5 mg/kg, i.p.) on the anti-immobility effect of AA in TST (A) and on locomotor activity in OFT (B).

3.1.3 Involvement of the synaptic protein PSD95 in the inhibitory effect of NLX on the antidepressant-like action of Ascorbic acid

The results presented in Figure 7 show the immunocontent of the PSD95 protein in the parahippocampal region (A) and prefrontal cortex (B) of animals injected with NLX (0.5 mg/kg, i.p.) and pre-treated with AA (1 mg/ kg, p.o.) using the western blot technique. Two-way ANOVA did not reveal significant changes in any of the treatments, either in the parahippocampal region (treatment with AA treatment with NLX, interaction of the two treatments (Figure 7A), and in the prefrontal cortex (treatment with Ascorbic acid, treatment with NLX, interaction of the two treatments (Figure 7B) of the animals on PSD95 immunocontent in the parahippocampal region (panel A) and prefrontal cortex (panel B) of mice. Values are expressed as mean +E.P.M. (N=6-8, parahippocampal region; N=4-6 cortex). The results were analysed by two-way ANOVA.



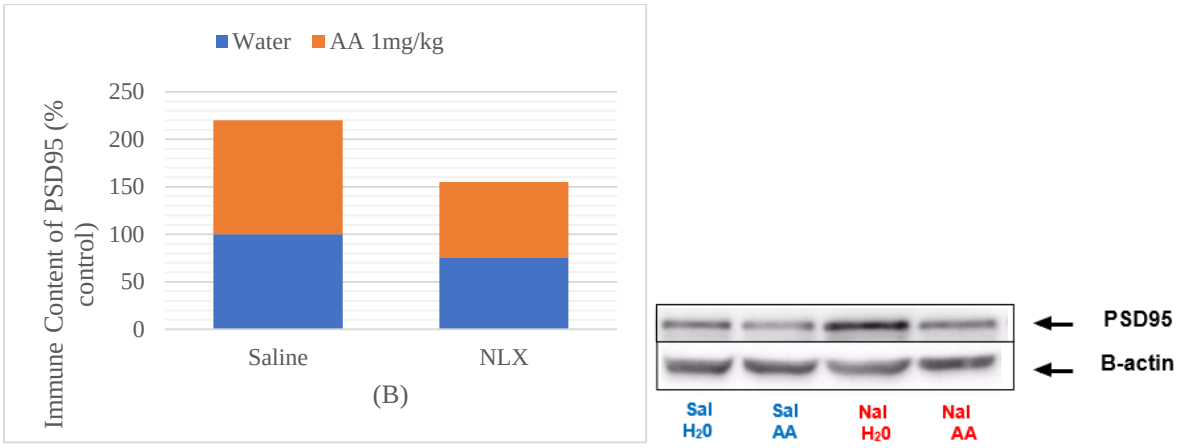


Figure 7: Effect of treatment with naloxonazine (5mg/kg, i.p.) and AA on PSD95 immunocontent in the hippocampus (panel A) and prefrontal cortex (panel B) of mice

3.2 Investigation of the effect of ascorbic acid on the model of mania induced by m-Amphetamine

3.2.1 Effect of repeated administration of AA in the model of mania induced by m-Amphetamine on locomotion and the number of movements of the animals in the PFT

Considering the evidence found in the literature that point to a possible antimanic effect of Ascorbic acid, in the second stage of this work, we studied the effect of AA in an animal model of mania induced by m-Amphetamine. Figure 8 shows the effect of repeated administration (14 days) of Lithium chloride in this model, on the number of crosses (A) of the animals in the PFT. Two-way ANOVA revealed significant differences for m-Amphetamine treatment, for treatment with Lithium chloride and for the interaction of m-Amphetamine treatment and Lithium chloride treatment. Post-hoc analysis showed that repeated administration of Lithium chloride for 14 days was able to prevent the increase in the number of crossings ($p<0.01$) caused by repeated administration of m-Amphetamine for 7 days ($p<0.01$). Figure 8B shows the repeated administration (14 days) of Lithium chloride, over the number of animal lifts in the PFT. Two-way ANOVA revealed significant differences for m-Amphetamine treatment, for treatment with Lithium chloride and for the interaction of m-Amphetamine treatment and Lithium chloride treatment. Post-hoc analysis showed that repeated administration of Lithium chloride for 14 days was able to prevent the increase in the number of withdrawals ($p<0.01$) caused by repeated administration of m-Amphetamine for 7 days ($p<0.01$).

Figure 9 shows the effect of repeated administration of AA for 14 days in the m-Amphetamine-induced mania model, on the number of mating (A) of the animals in the PFT. Two-way ANOVA revealed significant differences for m-Amphetamine treatment, but not for AA treatment or for the interaction of the

two treatments. Panel B shows the effect of repeated administration of AA for 14 days in the m-Amphetamine-induced mania model on the number of animal lifts at PFT, two-way ANOVA revealed significant differences for m-Amphetamine treatment and for interaction between m-Amphetamine treatment and AA treatment, but not for AA treatment. The post-hoc analysis showed that the repeated administration of m-Amphetamine ($p<0.01$) increased the number of crossings of the animals in the test. The treatment with AA was unable to revert this effect.

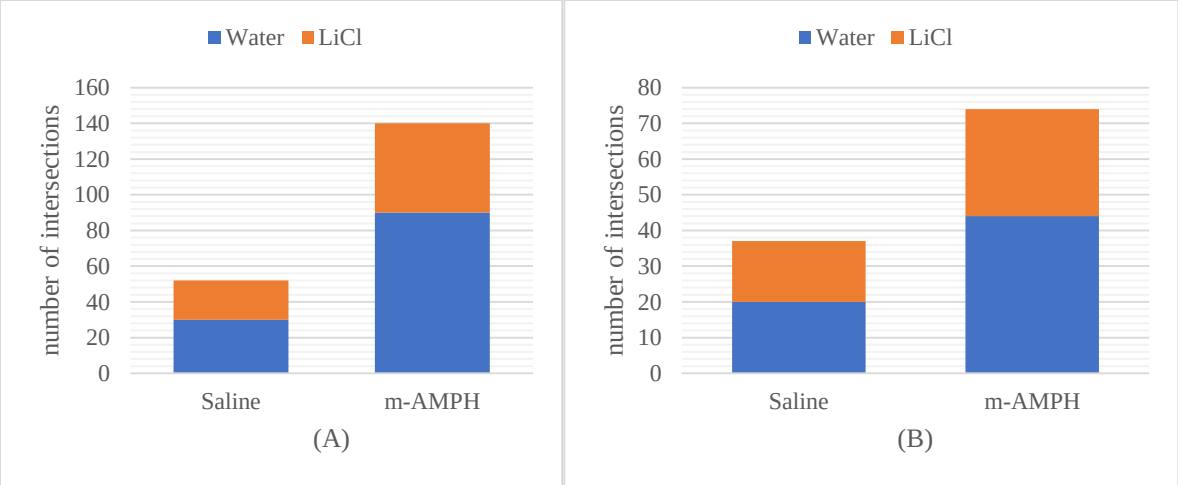


Figure 8: Effect of Lithium chloride in the m-Amphetamine-induced mania model on the number of crosses (A) and rearings (B) of animals in the PFT. Values are expressed as mean + E.P.M. (N=6-10).

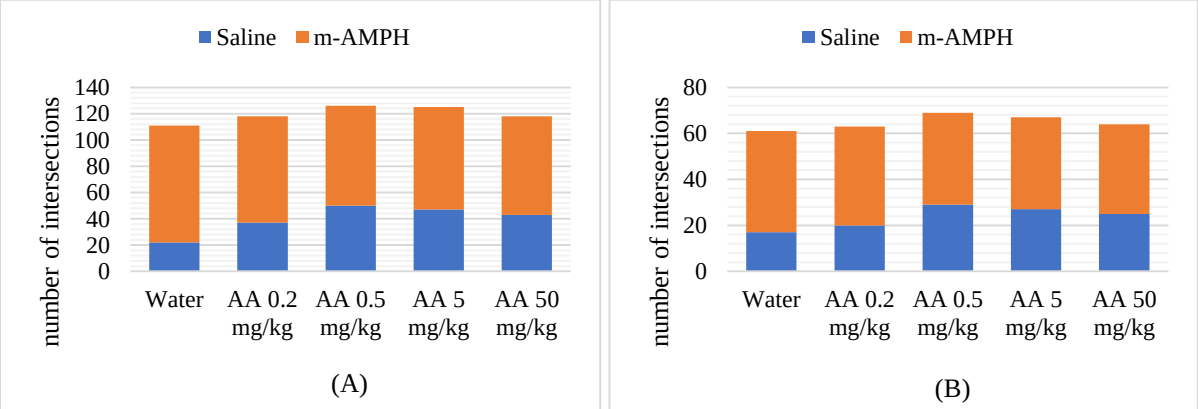


Figure 9: Effect of AA in the m-Amphetamine-induced mania model on the number of crosses (A) and readings (B) of animals in the PFT.

3.2.2 Influence of the model of mania induced by m-Amphetamine on the hippocampal and cortical immunocontent of B.D.N.F in rats treated or not with Lithium chloride or Ascorbic acid

The results presented in Figure 10 show the immunocontent of the neurotrophic factor B.D.N.F in the parahippocampal region (A) and prefrontal cortex (B) of the animals submitted to the mania protocol induced by m-Amphetamine using

the western blot technique. Densitometry followed by two-way ANOVA shows statistically significant differences for m-Amphetamine treatment, but not for AA treatment or Lithium chloride nor for the interaction of treatments in the animals' parahippocampal region (A). Two-way analysis of variance (ANOVA) revealed statistically significant differences in the prefrontal cortex between m-Amphetamine and AA treatments, as well as between the two treatments and their interaction or Lithium chloride, but not for treatment with AA or Lithium chloride.

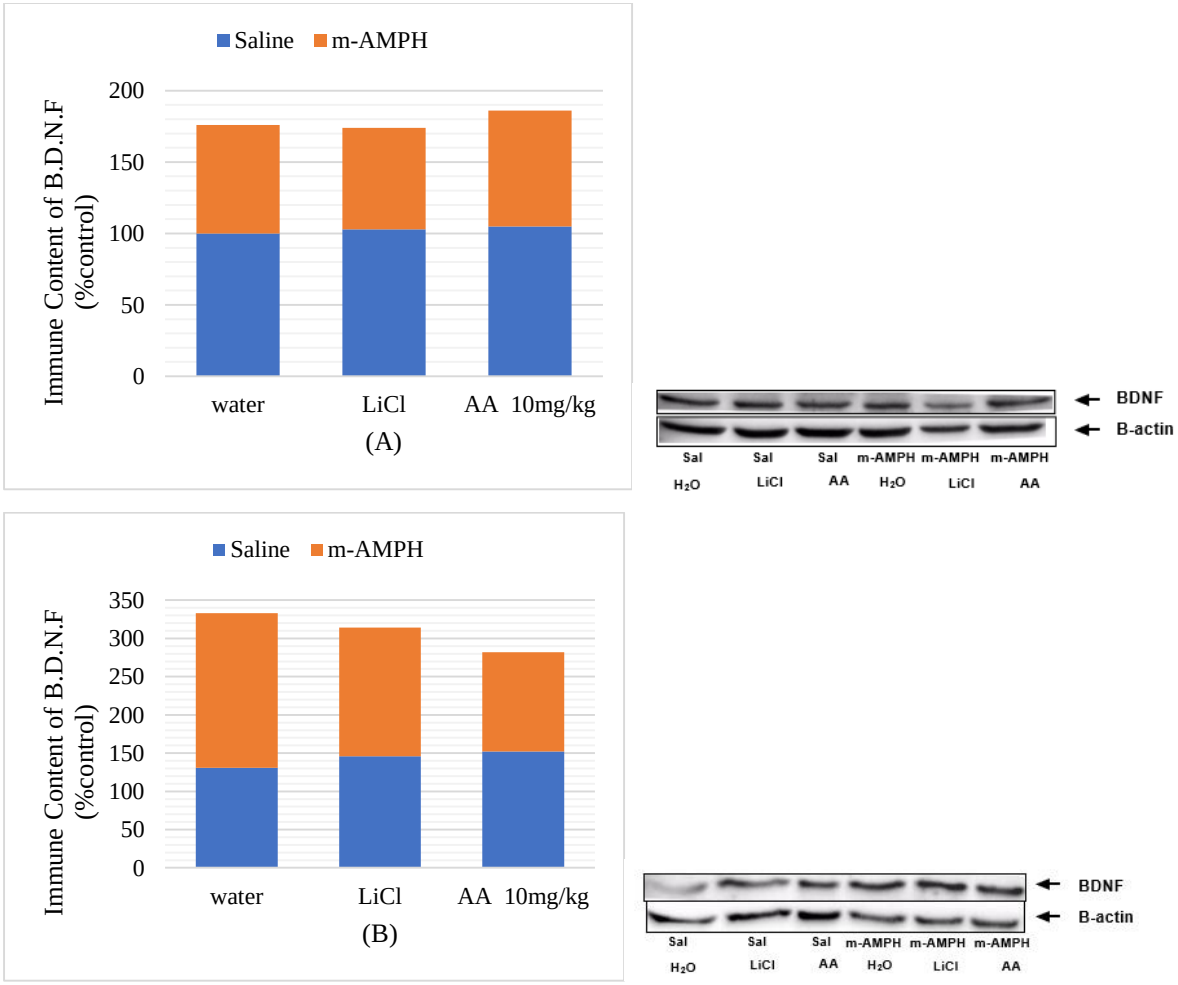


Figure 10. Effect of pre-treatment of animals with AA or Lithium chloride in the m-Amphetamine-induced mania model on B.D.N.F immunocontent in the parahippocampal region (A) and prefrontal cortex (B) of rats.

3.2.3 Influence of the m-Amphetamine-induced mania model on hippocampal and cortical FGF-2 immunocontent in rats treated or not with Lithium chloride or Ascorbic acid

The results presented in Figure 11 show the immunocontent of the growth factor FGF-2 in the parahippocampal region (A) and prefrontal cortex (B) of the animals submitted to the mania protocol induced by m-Amphetamine using the western blot technique. Analysis by densitometry followed by two-way

ANOVA did not reveal any statistical difference in any of the groups (treatment with m-Amphetamine, treatment with AA or Lithium chloride, treatment interaction in the animals' parahippocampal region. In the prefrontal cortex, two-way ANOVA detected significant differences in m-Amphetamine treatment, but not in treatment with AA or Lithium chloride or in the interaction of the two treatments.

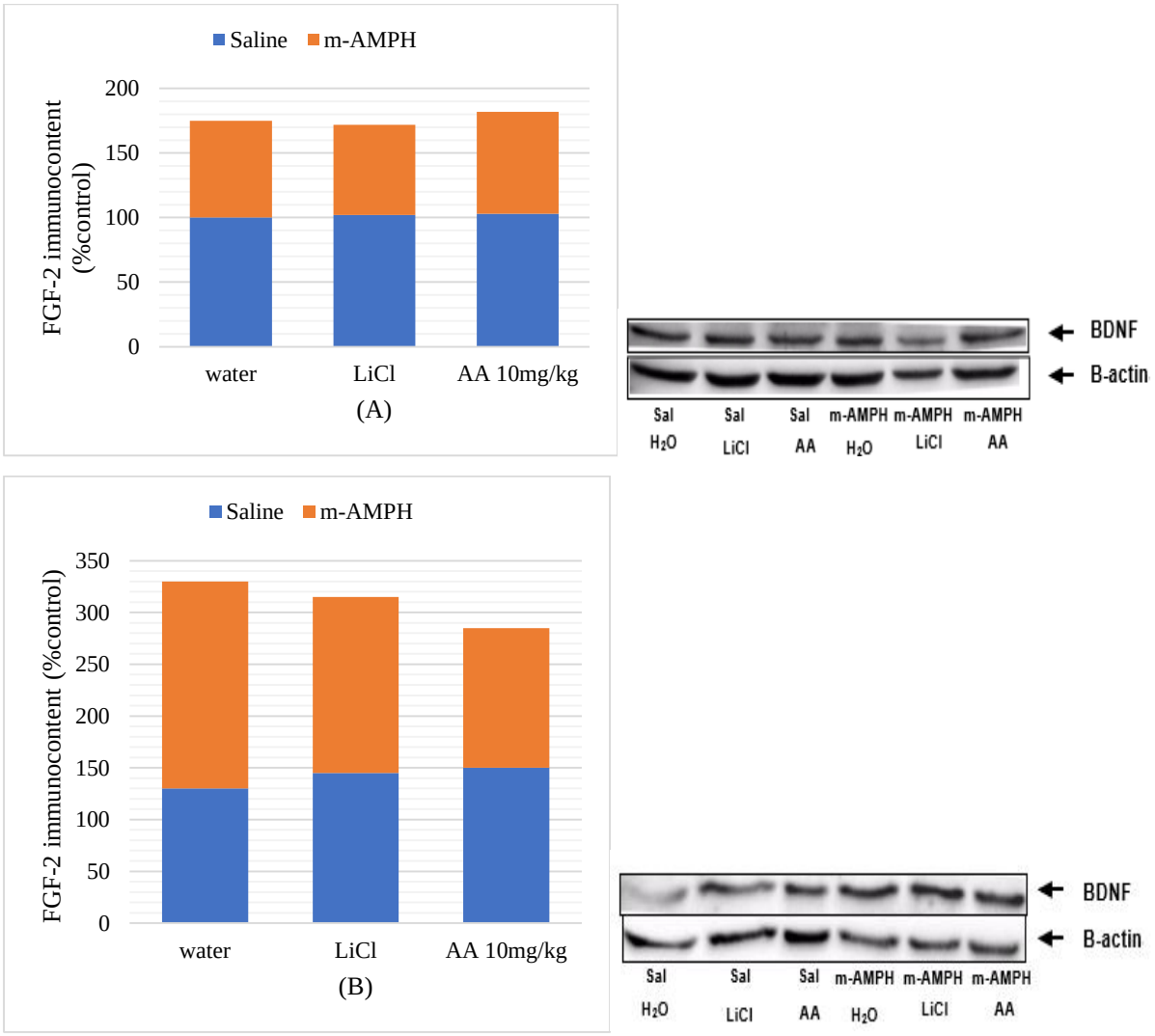


Figure 11. Effect of pre-treatment of animals with AA or Lithium chloride in the m-Amphetamine-induced mania model on FGF-2 immunocontent in the parahippocampal region (A) and prefrontal cortex (B) of rats.

4. DISCUSSION

Major depressive disorder (MDD) and bipolar disorder (BD) are two prevalent mood disorders. Both are costly to society, and we don't understand the neurochemical changes that generate them. Moretti, et al. (2018) hypothesised that opioid-stimulating antidepressants would be needed. Ascorbic acid's antidepressant potential has been explored. People with depression, with or without pharmacological treatment, have reduced plasma and serum ascorbic

acid levels, and supplementing with ascorbic acid helps improve depressive symptoms. AA supplementation increased fluoxetine's ability to reduce depressed symptoms. AA boosts antidepressant-like effects on the TST, a predictive test. Moretti. This data highlights the importance of studying ascorbic acid's antidepressant processes. In the first series of tests, naloxone was employed to investigate the influence of its administration on the effect of AA on the TST. The purpose was to determine if AA may promote an antidepressant effect depending on the opioid system. Naloxone blocks ascorbic acid's antidepressant effect, suggesting it requires opioid receptors. Experiments using agmatine, adenosine, and folic acid had comparable outcomes. Chemicals that impact animal locomotion may affect the TST, although no experimental groups demonstrated a change in PFT locomotion (Souza Brocardo, 2009).

Our data imply that Ascorbic acid's antidepressant-like effect involves 1-type opioid receptors. AA inhibits NMDA receptors, and NMDA receptor inhibition is related to endogenous opioid release, supporting the concept that ascorbic acid indirectly affects the opioid system through NMDA receptors and the opioid system. AA blocks NMDA receptors, increasing endogenous opioids that activate opioid receptors, especially 1-type. Despite these findings, ascorbic acid may bind directly to opioid receptors, especially the 1-subtype.

Morphine, an opioid agonist, may boost ascorbic acid's antidepressant effect on TST. This non-selective opioid agonist has an antidepressant-like impact on TST and TNF and synergizes with other antidepressants. (Schreiber, 2001) This investigation will offer information on the opioid system's function in ascorbic acid's antidepressant-like action. After discovering that ketamine's quick antidepressant effect is due to mTOR pathway activation, researchers have become aware of the need for more research into mTOR pathway signals. According to a study, activating □-opioid receptors can reduce A oligomer-induced neurotoxicity in cultured cortical neurons via mTOR signalling. Tramadol, an opioid-activating analgesic, increases ketamine's antidepressant-like effects through phosphorylating mTOR.

Moretti et al. (2020) showed that ascorbic acid's antidepressant-like effects require mTOR pathway activation. We explored PSD95's participation in the antidepressant-like effect of ascorbic acid and its interaction with the opioid system in mouse parahippocampal and prefrontal cortex homogenates. PSD95 immunocontent in parahippocampal tissues did not alter between groups, although it rose overall compared to the control. We attribute the absence of statistical significance to data variability, which will be validated by further studies. According to early studies, AA can increase PSD95 immunocontent in mice's parahippocampal area. Moreover, NLX has no influence on the probable rise in PSD95 immunocontent generated by Ascorbic acid, showing that the effect of NLX on TST, preventing the anti-immobility action of Ascorbic acid,

occurs via a mechanism independent of activation of mTOR and, therefore, PSD95. Despite Ascorbic acid's antidepressant-like action in this test, mTOR pathway activation appears unrelated to opioid system activation.

Our earlier research showed the serotonergic, noradrenergic, dopaminergic, and glutamatergic systems are involved in Ascorbic acid's antidepressant-like impact, as well as potassium channels and mTOR regulation. We know little about ascorbic acid's effects on BMD, especially during the manic phase. Older research show ascorbic acid affects the dopaminergic system. Perry, et al. (2006) studied dopaminergic chemicals in rats with a unilateral nigrostriatal lesion and amphetamine administration. Vitamin treatment stopped the observed circulation.

Pretreatment with ascorbic acid reduces amphetamine-induced hyperlocomotion in mice. Recent research shows that dopaminergic activation is required for Ascorbic acid's antidepressant-like effect on TST. Since BD is characterised by a pattern of changing dopamine levels, our findings imply that ascorbic acid may have a modulatory effect on the dopaminergic system, which might be helpful in treating BD.

The second phase of this investigation tested Ascorbic acid's antimanic effects in an animal model. After 7 days of m-Amphetamine therapy, hyperlocomotion in rats can be assessed by the number of times they cross the PFT and elevate their hind legs during the test (Moretti, et al. al., 2020). Our data showed that the m-Amphetamine-treated group outperformed the control group in both metrics. This hyperlocomotor impact could be averted by pre-treatment with Lithium chloride for 7 days and simultaneously treating with m-Amphetamine for 7 days. Studies show this. Oral dosages of 0.2, 0.5, and 50mg/kg ascorbic acid did not prevent behaviour changes. AA (0.2 and 0.5mg/kg, p.o.) has antidepressant-like effects.

We also studied the role of B.D.N.F and fibroblast growth factor-2 in the m-Amphetamine-induced mania paradigm. Studies suggest that B.D.N.F serum and plasma levels are lowered during the manic and sad phases but rebound to baseline during the euthymic phase. According to studies, B.D.N.F is a biomarker for mood disorders and therapy success. Many studies have established FGFs' importance in the brain, from neuronal development and repair to neural plasticity and learning. FGF-2 may cause emotional issues. FGF-2 mRNA expression is lower in depressive patients, while serum levels are higher during mania. m-Amphetamine dramatically modifies B.D.N.F in rat hippocampus homogenates. All groups treated with m-Amphetamine revealed a decrease in B.D.N.F in this brain region, consistent with the literature revealing that individuals with BD have decreased serum and plasma levels of BDNF during manic episodes. Similarly, d-AMPH therapy reduces parahippocampal BDNF immunocontent. m-Amphetamine increased the immunocontent of

B.D.N.F in the prefrontal cortex of rats, while AA inhibited this effect. According to the research, d-AMPH lowered the B.D.N.F immunocontent in this brain structure. In the setting of B.D.N.F activation, the two isomers of amphetamine cause opposite effects. (Presgraves, 2004)

The increase in B.D.N.F immunocontent in the m-Amphetamine-treated group may be a protective reaction to treatment-induced damage. AA blocked m-action, Amphetamine's perhaps due to a neuroprotective effect, obviating the need for BDNF upregulation. Pretreatment with lithium chloride prevented this. Tabebuia avellanedae extract prevents rise in hippocampus BDNF following olfactory bulbectomy in mice. Increased Bcl-2 immunocontent after restraint stress in mice presumably occurs from a similar mechanism. A study by Bücker et al. (2014) showed an increase in B.D.N.F plasma levels in traumatised children, suggesting that this increase is an effort to compensate for or neutralise childhood stress.

In the prefrontal cortex of the study rats, m-Amphetamine increased levels of the neurotrophin FGF-2. Our data show that FGF-2 mediates amphetamine's motor recovery after stroke Dysregulation of FGF-2 in persons with this syndrome causes poor brain growth and function. Bücker, et al. (2014) found that FGF-2 and other angioneurins are enhanced following manic episodes to provide neuroprotection.

Our findings add to research on ascorbic acid's antidepressant effect and mechanism of action. According to the behavioural criteria, animal model, and doses examined, this vitamin does not have an antimanic effect.

Conclusion

In animal models of stress-induced depression, restraint stress, and unpredictable chronic stress, ascorbic acid has been shown to have an antidepressant-like effect, with the compound having an effect similar to that obtained with fluoxetine treatment in reversing the depressogenic behaviour induced by these protocols. Lastly, a reduction in lipid peroxidation and a balance in endogenous antioxidant defences were associated with ascorbic acid's antidepressant-like effects in stress models. The ability of ascorbic acid to activate opioid receptors, specifically type 1 opioid receptor, accounts for its antidepressant effects. Additionally, ascorbic acid can activate the mTOR-PSD95 pathway without activating the opioid system. These findings further underscore the importance of the opioid system in the action of drugs with antidepressant activity and advance our understanding of how ascorbic acid exerts its antidepressant effects.

Despite the possibility that ascorbic acid has antimanic properties, the behavioural findings here do not corroborate this theory. AA did not have an antimanic effect in this paradigm or at the levels used, but it did protect the

brain from amphetamine's effects on the immunocontent of B.D.N.F in the prefrontal cortex. Our results suggest that ascorbic acid might be a different option for treating depression, while more investigation into this vitamin's antimanic effects is required.

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