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Inhibition of potassium sensitive adenosine triphosphate channels potentiates anti-depressant activity of creatine in mice

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ABSTRACT

Background: Depression is a mood disorder with poorly understood aetiology and treatment outcomes. Treatment with creatine, a nutraceutical associated with potassium sensitive adenosine triphosphate channels (K_{ATP}), produced improved results in preclinical studies of depression.

Objective: This study sought to investigate the role of K_{ATP} inhibitor and activator on the antidepressant activity of creatine in mice.

Methods: Four hundred and ninety male mice randomly allotted into groups of seven were either untreated (naïve) or pretreated with effective or sub-effective doses of glibenclamide or cromakalim, followed by selected doses of creatine, amitriptyline or imipramine for fourteen consecutive days. Groups of naïve and treated animals were thereafter subjected to forced swim test (FST) or tail suspension test (TST) or open field test (OFT).

Results: Pretreatment with effective or subeffective doses of glibenclamide and creatine, imipramine or amitriptyline significantly ($p<0.05$) reduced duration of immobility in the TST and FST. Conversely, cromakalim administered in combination with creatine, imipramine and amitriptyline antagonized the antidepressant effect of creatine, imipramine and amitriptyline. However, there was no difference in locomotor activity in the OFT across the treatment groups.

Conclusion: Inhibition of K_{ATP} channels potentiates the antidepressant activity of creatine.

Keywords: Creatine, K_{ATP}, antidepressant, cromakalim and glibenclamide

L'inhibition des canaux d'adénosine triphosphate sensibles au potassium potentialise l'activité antidépressive de la créatine chez la souris

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RÉSUMÉ

Contexte: La dépression est un trouble de l'humeur dont l'étiologie et les résultats du traitement sont mal compris. Le traitement par la créatine, un nutraceutique associé aux canaux adénosine triphosphate sensibles au potassium (KATP), a donné de meilleurs résultats dans les études précliniques de la dépression.

Objective: Cette étude a cherché à examiner le rôle de l'inhibiteur et de l'activateur du KATP sur l'activité antidépressive de la créatine chez la souris.

Méthodes : Quatre cent quatre-vingt-dix souris mâles réparties au hasard en groupes de sept ont été soit non traitées (naïves), soit prétraitées avec des doses efficaces ou sous-efficaces de glibenclamide ou de cromakalim, suivies de doses sélectionnées de créatine, d'amitriptyline ou d'imipramine pendant quatorze jours consécutifs. Des groupes d'animaux naïfs et traités ont ensuite été soumis à un test de nage forcée (FST) ou à un test de suspension de la queue (TST) et au tesst en plein champ (OFT).

Résultats : Un prétraitement avec des doses efficaces ou sous-efficaces de glibenclamide et de créatine, d'imipramine ou d'amitriptyline a réduit de manière significative ($p<0,05$) la durée d'immobilité dans le TST et le FST. Inversement, le cromakalim administré en association avec la créatine, l'imipramine et l'amitriptyline a antagonisé l'effet antidépresseur de la créatine, de l'imipramine et de l'amitriptyline. Cependant, il n'y avait pas de différence dans l'activité locomotrice de l'OFT entre les groupes de traitement.

Conclusion : L'inhibition des canaux KATP potentialise l'activité antidépressive de la créatine.

Mots clés : Créatine, KATP, antidépresseur, cromakalim et glibenclamide

INTRODUCTION

Major depressive disorder (MDD) is a common and severe neuropsychiatric disorder with an estimated disease burden of 150 million individuals per year; about 1 million people commit suicide annually and many of these deaths occur in young adults aged 15-29 years. The burden of MDD and depression-related suicide is likely to increase as a result of worsening socio-economic conditions and civil unrest, leading to internal displacement of persons and migration across state and international borders.[1-4]

Although underlying mechanisms of depression are now relatively better understood with the identification of certain biomarkers and the introduction of new classes of antidepressants, only 40-50% of patients on antidepressant therapy respond adequately to treatment. Furthermore, about 10-20% of patients on antidepressant therapy experience treatment resistant symptoms and are faced with difficulties in occupational and social activities, health deterioration and suicidal tendencies.[5-8] Adverse effects such as cognitive and mental impairment, sexual dysfunction, weight gain, sleep disturbances and attention deficits often result in poor adherence and consequent treatment failures.[9] These present enormous challenges in the management of depression, thus the need for novel and/or complementary strategies with maximum therapeutic benefit and minimal adverse effects cannot be overemphasized.

Strategies to improve efficacy of antidepressant therapy are currently being developed and explored; such strategies include augmentation of antidepressant therapy with synergistic agents such as nutraceuticals and therapeutic nutritional agents. Several nutrients such as zinc, folate, creatine and omega 3 which are known to play important roles in brain physiology and have been demonstrated to affect neurobiological process implicated in depression and neurodegenerative disorders are the subject of current research in various laboratories all over the world.[10-13]

Creatine (N-aminoiminomethyl-N-methylglycine), an endogenous guanidine compound synthesized from arginine, glycine and methionine in the liver, kidney, pancreas and brain is also acquired from high-protein foods such as fresh fish and meat. It facilitates generation of adenosine triphosphate in brain and muscle tissues and acts as a buffer for ATP/ADP stores.[14,15] Creatine, a nutraceutical has been demonstrated to play vital roles in various health and disease states and in brain bioenergetics. Functional impairment of creatine system resulted in deterioration in energy metabolism and creatine supplementation was found to be neuroprotective and beneficial in a variety of central nervous system disorders such as depression, stroke and Parkinson's disease. In animal models of depression, acute or chronic treatment with creatine reduced periods of immobility in the tail suspension test (TST), forced swim test and increased sucrose consumption in the sucrose preference test.[16-20] However, the mechanisms underlying these effects are yet to be clearly elucidated.[21]

Creatine kinase, which catalyzes conversion of creatine to phosphocreatine and consequent synthesis of ATP from ADP during periods of increased energy requirement, is associated with the ATP-sensitive potassium (K_{ATP}) channel.[22,23] The K_{ATP} comprising of two subunits viz inwardly rectifying potassium channel Kir6.1 or 2 and the regulatory subunit sulfonylurea receptor SUR (SUR1 or SUR2) are strongly expressed in the hippocampus, prefrontal cortex, amygdala, and hypothalamus and are implicated in the pathophysiology of depression.[24-27] In preclinical studies, K_{ATP} channel inhibitors such as glibenclamide were found to augment the antidepressant effects of serotonin or noradrenaline reuptake inhibitors while K_{ATP} channel activators, such as minoxidil and cromakalim, increased indices typical of depression in mice.[27-29]

In this study, we seek to investigate the role of K_{ATP} in the antidepressant action of creatine in murine models of depression, using inhibitors and activators of the K_{ATP} channel.

MATERIALS AND METHODS

Animals

Four hundred and ninety male albino mice weighing 20-25 g were used for this study. They were housed in groups of seven animals per cage, exposed to natural light and dark cycles, and maintained at room temperature. Mice were acclimatized for one week before the commencement of the study and allowed free access to standard laboratory food and clean water ad libitum throughout the duration of the study. Adequate hygiene was maintained through daily cleaning of cages. Experiments were conducted between 8.00 a.m. and 4.00 p.m. All procedures in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.[30] Ethical approval was obtained from the Ethics Committee of Faculty of Pharmacy, University of Benin, Nigeria (EC/FP/019/25).

Drugs

Creatine monohydrate (Dymatize®, USA), amitryptyline (TEVA, UK), Imipramine (TEVA, UK), glibenclamide (Sanofi Avensis), Cromakalim (Sigma Aldrich, USA).

Tail suspension test (TST)

The TST test is based on the fact that animals subjected to the short-term inescapable stress of being suspended by their tail, will develop an immobile posture. Mice were suspended individually 50 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded by observers unaware of drug treatment over a six-minute period.[31] Mice were considered immobile only when they hung passively and completely motionless.

Forced swim test (FST)

In the FST, animals were placed in an open cylinder-shaped flask (diameter 10 cm, height 25 cm) filled with water and allowed to swim for six minutes. The FST is used to evaluate behavioural immobility of mice as a selective standard animal paradigm for assessing antidepressant activity. Mice subjected to the FST are regarded as immobile when they stop struggling and float motionless in the water, only making movements necessary for keeping their head above water and prevent drowning. Periods of immobility in the last four minutes of the test were recorded by observers blinded to the treatment. After each test, the mice were dried and returned to their cages. A sieve was also used to remove animal droppings from the water and the water was changed intermittently.[32,33]

Open field test (OFT)

To assess the possible effects of test drugs on locomotor activity, mice were evaluated in the open-field apparatus. Mice were placed individually in a wooden box (40 cm×60 cm×50 cm) with the floor divided into twelve equal squares. The number of squares crossed by the mice with its four paws was registered during a period of 5 minutes by unbiased observers.[34,35]

Drug treatment

In the first phase of this study, forty-nine mice randomly distributed into seven groups of seven animals (groups 1-7) were used. The mice in group 1 were not subjected to any drug treatment (naïve) while those in groups 2-7 were treated with glibenclamide intraperitoneally (i.p) at a dose of 3 mg/kg.[27] Fifteen minutes later, they were treated with the following drugs: vehicle (group 2), 0.01 mg/kg creatine (group 3), 0.1 mg/kg creatine (group 4), 1 mg/kg creatine (group 5), 25 mg/kg imipramine (group 6) and 10 mg/kg amitriptyline (group 7) orally. This treatment was carried out for 14 days; thereafter animals were subjected to the FST as previously described.

Separate groups of ninety-eight mice (n=7 per group) received the same treatment regimen as described above; forty-nine were subjected to the TST and the other forty-nine to the OFT as previously described.

In the second phase of the study, separate groups of animals (n=147) were randomly distributed into groups of seven animals, groups 1-7. The mice in group 1 were not administered any drug and served as naïve control. Those in groups 2-7 were administered 1 mg/kg cromakalim i.p followed fifteen minutes later by vehicle (group 2) 0.01 mg/kg creatine (group 3), 0.1 mg/kg creatine (group 4), 1 mg/kg creatine (group 5), 25 mg/kg imipramine (group 6), 10 mg/kg amitriptyline (group 7) orally. After daily treatment for 14 days, the animals were used subjected to the TST, FST or OFT.

In the third and fourth phases of the study, following the methods described in other studies[27,36-37] with some modifications, sub effective doses of glibenclamide (1 mg/kg), cromakalim (0.1 mg/kg), amitriptyline (1mg/kg) and imipramine (2.5 mg/kg) were used.

In phase three, naïve mice, group 1 did not receive any drug treatment while the mice were treated with glibenclamide and fifteen minutes later with vehicle (group 2), 0.01 mg/kg creatine (group 3), 0.1 mg/kg creatine (group 4), 1 mg/kg creatine (group 5), 2.5 mg/kg imipramine (group 6), and 1 mg/kg amitriptyline (group 7) orally. This treatment regimen was carried out daily for 14 days after which the animals were subjected to the TST or FST

In the final phase of this study, naïve mice were used as naïve control and were not administered any drug while other groups of mice were treated with 0.1 mg/kg cromakalim and fifteen minutes later with vehicle (group 2), 0.01 mg/kg creatine (group 3), 0.1 mg/kg creatine (group 4), 1 mg/kg creatine (group 5), 2.5 mg/kg imipramine (group 6), and 1 mg/kg amitriptyline (group 7) via the oral route. This treatment regimen was carried out for 14 days after which forty-nine mice from groups 1-7 were subjected to the TST or the FST as previously described.

Statistical analysis

The results were analyzed for statistical significance, using One-Way Analysis of Variance (ANOVA) followed by Tukey post hoc test using Sigma stat ® version 14.5. A difference of p-values less than 0.05 ($p < 0.05$) was considered significant. The results are presented as mean ± standard error of mean (SEM).

RESULTS

Effects of Treatment with Effective Doses of Glibenclamide in the FST, TST and OFT

Pre-treatment with glibenclamide 3 mg/kg significantly ($p<0.05$) reduced periods of immobility in the TST and FST at all dose levels of creatine, imipramine and amitriptyline treated animals when compared to the naïve and vehicle treated animals. There was however no significant difference in locomotor activity across all treatment groups in the OFT. Data is presented in Fig 1A-C.

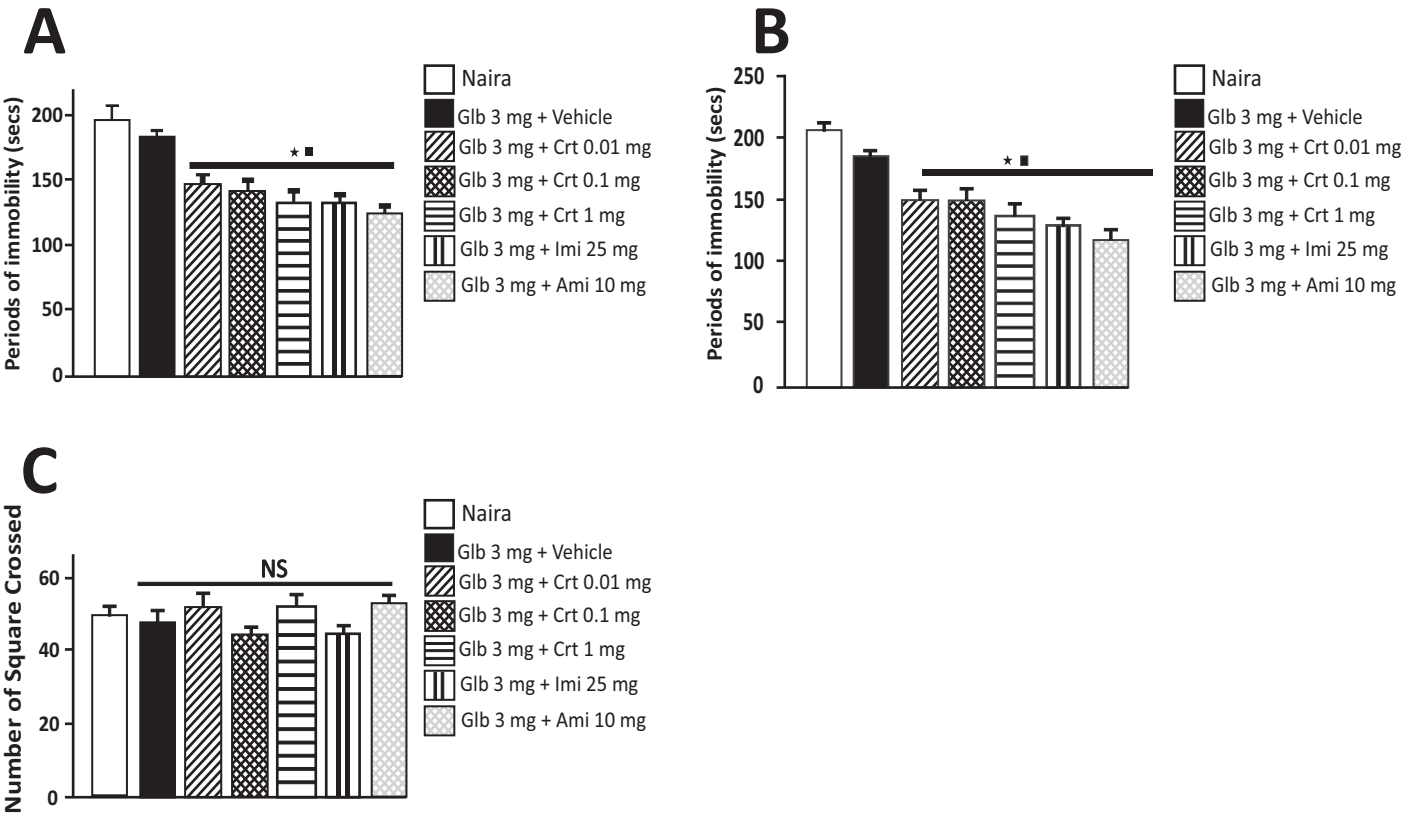

Fig 1: Effect of pretreatment with 3mg/kg Glibenclamide in the TST, FST and OFT.

Fig 1 shows the effects of pretreatment with effective doses of glibenclamide on creatine, amitriptyline and imipramine treated mice analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Treatment with glibenclamide significantly reduced periods of immobility in the TST and FST (A & B respectively) but did not affect locomotor activity (C). Data is presented as mean ± SEM., *$p < 0.05$ compared with naïve group, # $p<0.05$ compared to the vehicle group, NS, not significant; n = 7 per group. Glb indicates glibenclamide; Crt, creatine; Imi, imipramine, Amy, amitriptyline

Effects of treatment with effective doses of cromakalim in the FST, TST and OFT

Pre-treatment with cromakalim 1 mg/kg did not significantly reduce periods of immobility in both the TST and FST at all dose levels of creatine, imipramine and amitriptyline treated animals when compared to the naïve and vehicle groups. There was also no significant difference between all treatment groups in the OFT. Data is shown in Fig 2A-C.

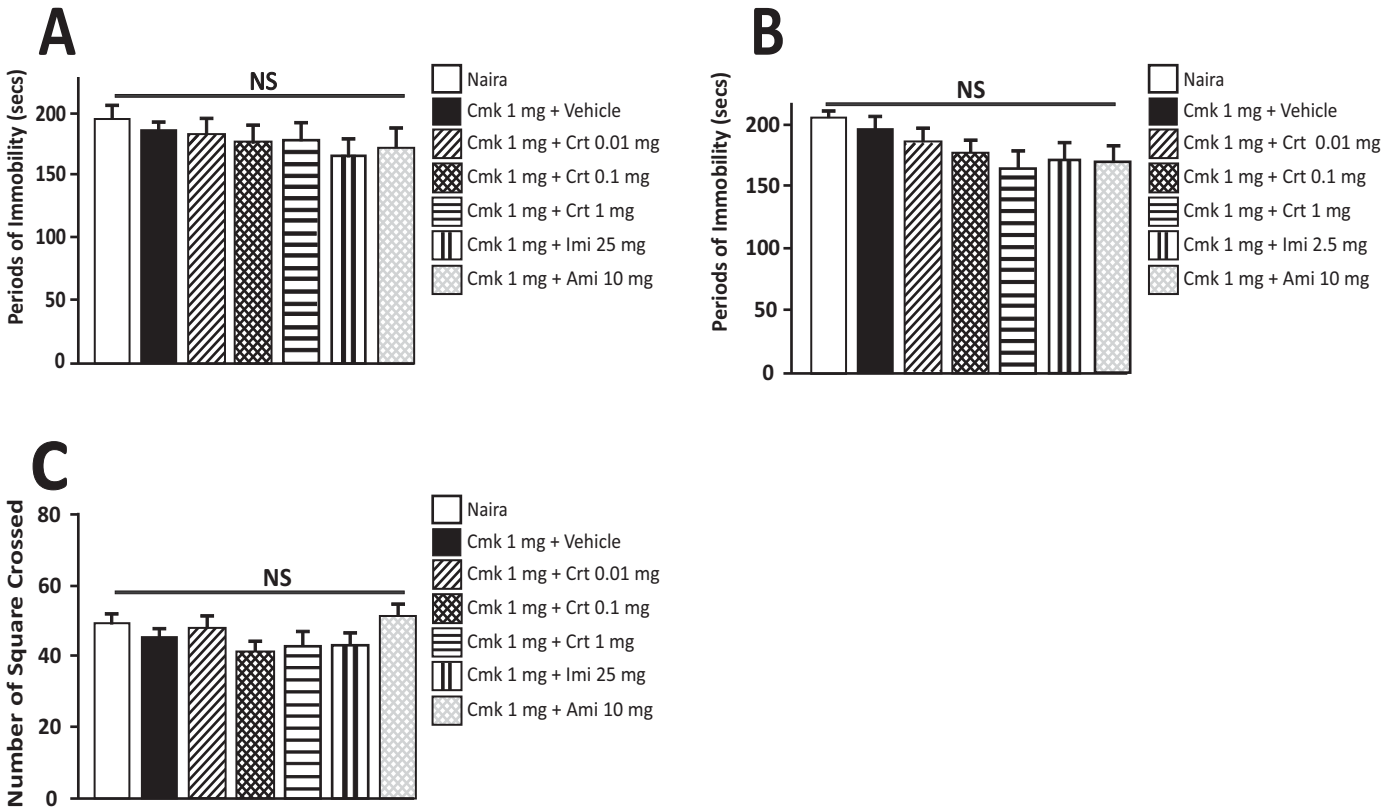

Fig 2: Effect of pretreatment with 1mg/kg cromakalim in the TST, FST and OFT.

Fig 2 shows the effects of pretreatment with effective doses of cromakalim on creatine, amitriptyline and imipramine treated mice. Treatment with cromakalim did not reduce periods of immobility in the TST and FST (A & B respectively) nor affect locomotor activity (C). Data is presented as mean ± SEM. NS, not significant; n = 7 per group. Cmk indicates cromakalim; Crt, creatine; Imi, imipramine, Amy, amitriptyline. Results were analyzed using ANOVA followed by Tukey post hoc test.

Effects of Sub-effective Doses of glibenclamide, imipramine and amitriptyline in the FST and TST

Pre-treatment with glibenclamide 1 mg/kg significantly ($p<0.05$) reduced periods of immobility in the TST and FST in creatine, imipramine and amitriptyline treated animals when compared to the naïve and vehicle groups.

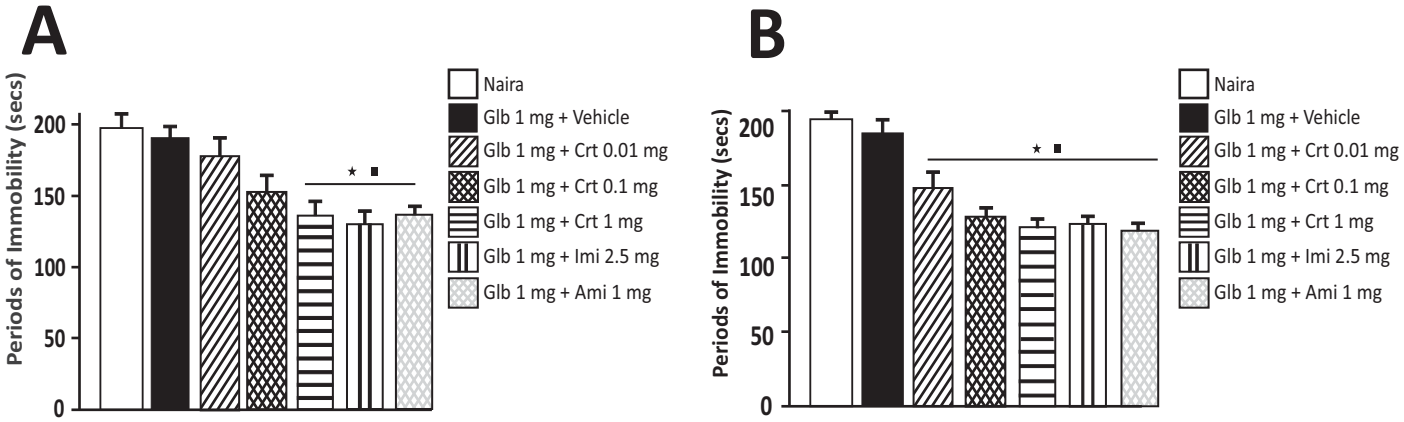

Fig 3: Effect of pretreatment with 1 mg/kg glibenclamide in the TST, FST and OFT.

Statistical analysis (ANOVA followed by Tukey post hoc test) of periods of immobility in the TST and FST following pre-treatment with sub-effective doses of glibenclamide, amitriptyline and imipramine. High dose creatine, imipramine and amitriptyline resulted in significant reduction in periods of immobility in the TST (A) while all doses of creatine and subeffective doses of imipramine and amitriptyline reduced immobility in the FST (B).

Data is presented as mean ± SEM. *p < 0.05 compared with naïve group, # p<0.05 compared to the vehicle group; n = 7 per group. Glb represents Glibenclamide; Crt, creatine; Imi, imipramine, Amy, amitriptyline

Effects of Sub-effective doses of cromakalim, imipramine and amitriptyline in the FST and TST

Pre-treatment with 0.1 mg/kg Cromakalim did not significantly reduce periods of immobility in the TST in treatment groups except at the highest dose of creatine when compared to the vehicle group. However, the highest dose of Creatine (1 mg/kg), Imipramine and Amitriptyline resulted in significant reduction (p<0.05) in periods of immobility in the FST.

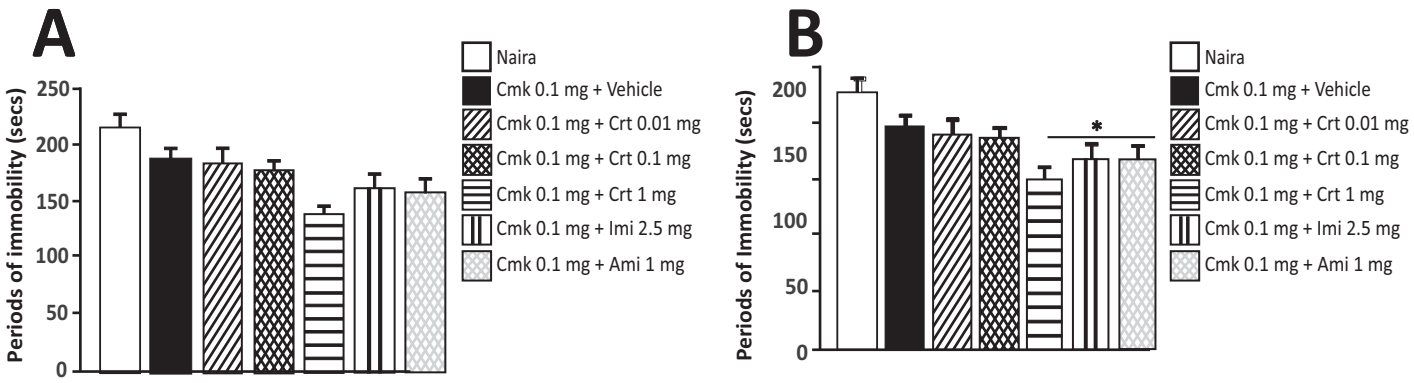

Fig 4: Effects of pretreatment with 0.1 mg/kg cromakalim on TST and FST

Statistical analysis of periods of immobility in the TST and FST following pre-treatment with sub-effective doses of cromakalim, amitriptyline and imipramine using ANOVA followed by Tukey post hoc test. Creatine 1 mg significantly reduced immobility in the TST (A) while 1mg/kg Creatine, imipramine and amitriptyline resulted in significant reduction in periods of immobility (B). Data is presented as mean ± SEM, * p<0.05 compared to the vehicle group; n = 7 per group. Cmk represents cromakalim; Crt, creatine; Imi, imipramine, Amy, amitriptyline

DISCUSSION

In this study, pre-treatment with glibenclamide reduced immobility in the TST and FST suggestive of augmented antidepressant activity of creatine while therapeutic doses of cromakalim did not reduce immobility in both the TST and FST indicative of depression in these models.

The FST and TST are widely used animal models of depression as a result of their sensitivity to different classes of clinically useful antidepressants such as selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors and the atypical antidepressants.[27,32] The TST and FST are based on the principle that animals develop immobile postures when subjected to inescapable stress of being suspended by their tails or placed in an inescapable cylinder of water. This stress induced immobility simulates clinical depression and clinically useful antidepressants have been found to reduce periods of immobility.[31, 38-40]

Creatine has been demonstrated to have antidepressant properties and mechanisms of action such as modulation of dopaminergic pathways and interaction with 5-HT1A receptors in the brain have been described.[17,18,37] Pre-treatment with effective and sub-effective doses of K_{ATP} channel antagonist (glibenclamide) potentiated both effective and sub-effective doses of creatine, amitriptyline and imipramine. In similar studies, inhibition of K_{ATP} channels enhanced basal release of serotonin in rat hippocampal slices[41] and inhibited membrane hyperpolarization, Ca^{2+} influx, and

consequent excitatory response.[42,43]

To substantiate our hypothesis, mice were treated with the K_{ATP} channel opener cromakalim. Pretreatment with cromakalim resulted in increased periods of immobility in mice treated with creatine in the FST and TST. However, in mice treated with sub-effective doses of cromakalim and subeffective doses of creatine the antidepressant effect of creatine was not evidenced in both models of depression with low doses of creatine. In similar studies K_{ATP} channel openers reversed the antidepressant effects of various drugs, such as imipramine, amitriptyline, desipramine, and paroxetine in the FST.[39] Inhibition of active K_{ATP} channels might elicit more depolarized membrane potentials, Ca^{2+} influx and result to an increase in the electrical activity in these neurons, which could subsequently exert antidepressant-like behaviors.[42,43] Taken together, our result lends credence to our assumption that the antidepressant-like effect of creatine may involve the K^+ channels. Molecular and cellular studies would provide more insights to possible mechanisms of action.

To demonstrate if the reduction in the periods of immobility elicited by glibenclamide and creatine were due to the psycho-stimulant activity of these compounds, animals were subjected to the OFT. Psycho-stimulant compounds can induce hyperactivity, resulting in increased ambulatory behaviour in the OFT and increased periods of immobility in the TST and FST, giving false positive impressions of antidepressant activity of test compounds.[44] The result obtained from the OFT in the present study indicates that neither creatine alone nor the combination with K_{ATP} channel inhibitor or opener and other tricyclic antidepressants altered locomotor activity. Therefore, the observed synergistic effect seen with creatine in combination with the K_{ATP} channel inhibitor, glibenclamide is not attributable to hyper locomotor activity of any of these compounds.

This study shows for the first time the role of glibenclamide in potentiating increased mobility of mice in the TST and FST produced by treatment with creatine.

CONCLUSION

This study suggests that the antidepressant effect of creatine is mediated by the inhibition of K_{ATP} channel. However, further investigations aimed at elucidating the underlying molecular mechanisms are required.

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