Original Article

Antidepressant-like Effect of Vitamin B6 in Mice Forced Swimming Test and the Possible Involvement of the Noradrenergic System

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Abstract

Background: Vitamin B6 is a cofactor for various enzymes that are involved in neurotransmitter production. It has been shown that vitamin B6 administration reduces immobility time in mice forced swimming test (FST), which suggests potential antidepressant activity in humans. The aim of this study was to observe the possible involvement of the noradrenergic system in the antidepressant effects of vitamin B6 during FST in mice. **Material and methods:** Each of the following drugs was administered with vitamin B6: a tricyclic antidepressant (imipramine), α1 adrenoceptor antagonist (prazosin), α2 adrenoceptor antagonist (yohimbine), and β adrenoceptor antagonist (propranolol) and α-methyl-*p*-tyrosine (AMPT), a selective inhibitor of the enzyme tyrosine hydroxylase. **Results:** The antidepressant effect of vitamin B6 (100 mg/kg) was increased by adding imipramine (5 mg/kg), prazosin (1 mg/kg) to the treatment and slightly by propranolol (2 mg/kg). Yohimbine (1 mg/kg), to some extent, reversed vitamin B6 effects although not completely compared with the control group, whereas AMPT (100 mg/kg) administration absolutely reduced vitamin B6 antidepressant effect during the FST. **Conclusion:** This study provides evidence indicating that the antidepressant-like effect of vitamin B6 in the FST is dependent on its interaction with α and β adrenoceptors and the noradrenergic system plays a critical role in its antidepressant benefits.

Keywords: *Adrenoceptor, depression, forced swimming test, noradrenergic system, vitamin B6*

Introduction

Depression is a mood disorder connected with high incidence of relapse, chronicity, recurrence, psychosocial damage, and suicide.[1] According to the monoaminergic hypothesis, the monoaminergic system is one of the most important targets in the pathophysiology of depression as the dysfunction of monoaminergic circuits in the central nervous system causes depression.[2] This theory is supported by a great number of neurochemical findings[3] and by the successful treatment of depressed individuals with classical antidepressant drugs. These drugs have been used for decades and enhance monoaminergic neurotransmissions such as the monoamine oxidase inhibitors and the tricyclic antidepressants (TCA).[4] Dietary interventions and vitamin supplementation, especially the B vitamins, are among the most popular alternative medications for depression.[5]

Vitamin B6 vitamers are rapidly oxidized in the liver to pyridoxal and phosphorylate to pyridoxal 5-phosphate (PLP), which is the main circulating vitamer. The free PLP that is not bound to enzymes is dephosphorylated and oxidized to pyridoxic acid, which is the main urinary metabolite of the vitamin.[6] Vitamin B6 is one alternative treatment that could alleviate depression disorder through its role in the metabolism of neurotransmitters that are considered to be important in the manifestation of depression.[6]

Monoamine biosynthesis is reliant on two metabolic pathways: dihydroxyphenylalanine and 5-hydroxytryptophan (5-HTP) that are converted by aromatic l-amino acid decarboxylase (AADC) to dopamine and serotonin, respectively.[7] Dopamine is a neurotransmitter and it is the precursor for noradrenaline (NA) and adrenaline. Vitamin B6 is the cofactor for AADC,[6] and it was observed earlier that vitamin B6 administration reduces immobility time in mice forced swimming test (FST), which indicates its possible antidepressant activity in humans.[8] There are well-known evidence regarding the role of noradrenergic system in depression; antidepressants (such as doxepin, amitriptyline, and nefazodone)

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have a noticeable affinity for α1-adrenoceptors (ARs), and mirtazapine enhances NA release by directly blocking presynaptic α2-ARs.[9] Dopa decarboxylase compared with the enzymes 5-HTP decarboxylase and glutamate decarboxylase has a higher affinity for vitamin B6.[10]

This study was aimed to extend the previous findings regarding the antidepressant-like effect of vitamin B6 in mice FST in several ways: first, to evaluate its antidepressant effect following using low dose of imipramine (a TCA); second, to observe the changes in depressive behavior following the administration of α1-AR antagonist (prazosin), α2-AR antagonist (yohimbine), and β-AR antagonist (propranolol); and finally, to observe the change in behavior after administrating α-methyl-*p*-tyrosine (AMPT), a selective inhibitor of the enzyme tyrosine hydroxylase.

Materials and Methods

Animals

Male albino mice weighing 28 ± 4 g were maintained at 21°C ± 2°C with free access to water and standard mice chow, under a 12:12 h of light:dark cycle (the lights were on from 6 am to 6 pm). Each experimental group comprised six animals that were housed per cage and they were placed in the experimental room 24 h before the test for acclimatization. All the experiments were performed between 8 am and 1 pm in the pharmacology laboratory. All animal procedures were performed in accordance with the National *Guide for the Care and Use of Laboratory Animals* issued by the Isfahan University of Medical Sciences (ethical no.: IR.MUI.REC.1396.3.750). All the efforts in the experiments were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Locomotor test

The locomotor activity should be tested before psychological tasks as variations in locomotor activity nonspecifically affect performance in many behavioral paradigms. The motor activity of mice was assessed in an open arena (Borj Sanat, Iran) divided by red beams into 15 zones in a 5 × 3 grid formation. Mice were placed facing toward the wall and were allowed to explore the field for 3 min. By passing animals through the beams, the number of zone entries was counted automatically, whereas rears on hind legs were recorded manually. Finally, total activity for each animal was calculated, which was the sum of zone entries (horizontal exploration) and rears (vertical exploration).

Forced swimming test

After the locomotor test, depression was assessed in mice by the FST.[11] Mice were forced to swim in 25°C water in a glass beaker (diameter 12.5 cm) for 6 min and the measurements were carried out in the last 4 min of the test. The depth of water was about a level that the mice could neither touch the bottom of the container with their paws or tail nor could they escape it (12 cm). The behaviors selected for measurement in the modified FST were climbing behavior, defined as the time animal intends to climb the glass beaker; swimming behavior, defined as horizontal movement throughout the beaker, which involved at least two limbs and included crossing across quadrants of the cylinder; and immobility, measured when no additional activity was observed other than that required to keep the head of the mouse above the water.[12] The whole experiment was recorded by a camera and analyzed later. After 6 min, the mice were dried carefully and returned to their home cage.

Drugs administration

Vitamin B6 (100 mg/kg) (pyridoxine HCl, 200 mg/mL, Caspian Tamin, Tehran, Iran) was injected intraperitoneally (IP) for 6 consecutive days,[8] whereas the corresponding control animals received normal saline. Yohimbine (Sigma, Mumbai, India; 1 mg/kg, IP), prazosin (A gift from Amin Industry, Isfahan, Iran; 1 mg/kg, IP), imipramine (Sigma, Steinheim, Germany; 5 mg/kg, IP), and propranolol (1 mg/mL, Polfa Warszawa S.A., Poland; 2 mg/kg, IP) were all diluted in normal saline and the doses were in accordance with previous studies.[13,14] They were injected on day six 30 min before vitamin B6 final dose. AMPT (Sigma, Mumbai, India; 100 mg/kg, IP) solution was freshly prepared in Dimethyl sulfoxide (DMSO) solution (0.1%) and administered 4 h before the final dose of vitamin B6.[15] The vehicle group received the relevant vehicle as the result for vehicle DMSO solution (0.1%) was not different from that of the normal saline group; hence, it is not reported separately. All the injections were adjusted for 10 mL/kg mice body weight.

Data processing and statistical analysis

Results were expressed as group mean value ± standard error of the mean (SEM). All results were analyzed by one-way analysis of variance, followed by Tukey's multiple comparison tests. *P* values less than 0.05 were considered significant. The software programs used for data analyzing and making graphs were Excel 2010 (El Paso, Texas, USA) and the GraphPad Prism 6 (San Diego, California, USA).

Results

Effect of different drugs on behaviors during the forced swimming test

As shown in Figure 1A, the drugs alone administered did not change immobility during the FST compared with the control group (180 ± 4.6 s). Thus, the selected doses did not change the depressive behavior. The mobile phase was different, although imipramine significantly increased the swimming time (83 ± 7.6 s vs. control 55 ± 5 s) [Figure 1B], the climbing time was considerably higher for propranolol and AMPT (31 ± 7 s and 37 ± 7 s, respectively, vs. control 1.6 ± 1 s) [Figure 1C]. Table 1 shows that prazosin caused less horizontal and therefore less locomotor activity compared with the control group, but the doses used here do not cause sedation as the one sample *t*-test shows a significant difference from zero ($t = 6.5$, df = 5; $P < 0.001$). Therefore, the drugs' doses used here neither cause sedation nor stimulation, thus the changes in the immobility observed during the FST were considered as the effects of the drugs on depressive behavior.

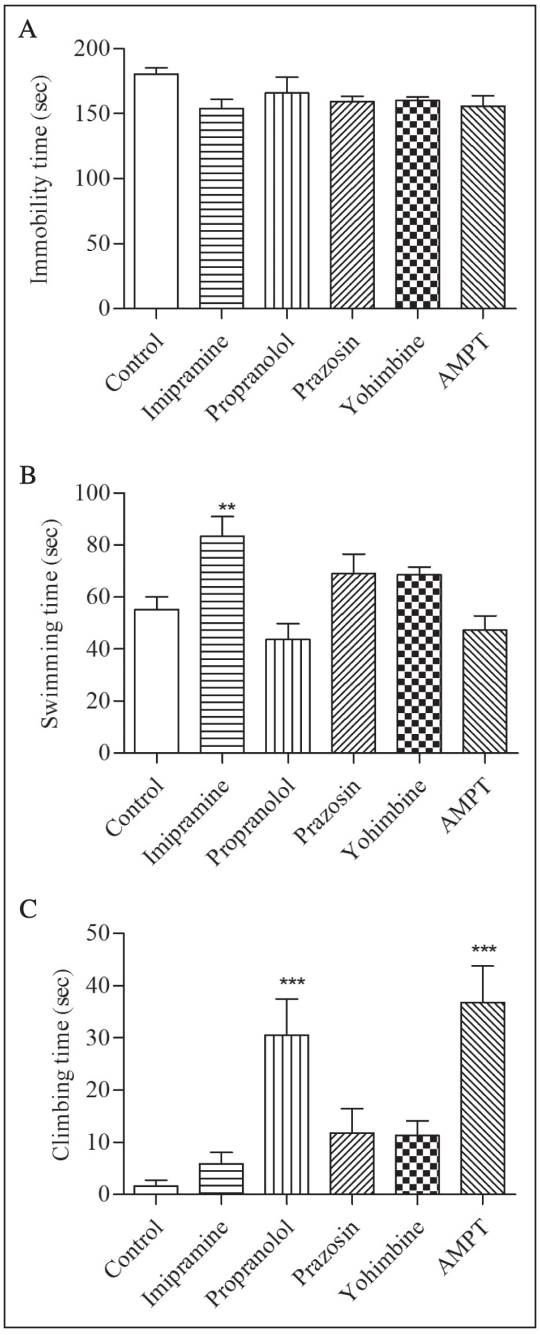

Figure 1: Effect of the drugs alone on behavior—(A) the immobility time, (B) the swimming time, and (C) the climbing time—during the forced swimming test. Imipramine (5 mg/kg), propranolol (2 mg/kg), prazosin (1 mg/kg), yohimbine (1 mg/kg), and α-methyl-*p*-tyrosine (AMPT, 100 mg/kg) were all administered intraperitoneally before testing. Number of animals in each group was six, control animals received normal saline. Results are expressed as group mean ± standard error of mean and analyzed by analysis of variance followed by Tukey's comparison tests. ***P* < 0.01 and ****P* < 0.001 compared with the control group

Effect of different drugs on vitamin B6-induced anti-immobility effect during the forced swimming test

As it could be observed in Figure 2A, vitamin B6 alone (vitamin B6 + vehicle) significantly reduced the immobility time during the FST, which indicates its antidepressant effect

Table 1: Effect of the drugs on the locomotor test

Treatment groups	Horizontal movements	Vertical movements	Total activity
Control	113 ± 6.8	10.9 ± 2.9	123.9 ± 9.5
Vitamin B6	127.5 ± 5.5	34 ± 4***	162 ± 9
Imipramine	132 ± 18	18.3 ± 3.2	150.8 ± 21
Propranolol	82.3 ± 6.7	2.5 ± 0.4*	84.8 ± 7
Prazosin	55 ± 10***	3.2 ± 1.7	81.6 ± 34**
Yohimbine	101 ± 10	7.6 ± 2	108.8 ± 11.6
AMPT	116.5 ± 10	9.5 ± 3	110.8 ± 19

**P* < 0.05, ***P* < 0.01, and ****P* < 0.001 compared with the control group. Total animal activity is the sum of horizontal and vertical movements. Vitamin B6 (100 mg/kg, IP) was administered for 6 consecutive days, imipramine (5 mg/kg), propranolol (2 mg/kg), prazosin (1 mg/kg), yohimbine (1 mg/kg), and α-methyl-*p*-tyrosine (AMPT, 100 mg/kg) were all administered IP before the test. Number of animals in each group was six, control animals received normal saline. Results are expressed as group mean ± standard error of mean (SEM) and analyzed by analysis of variance (ANOVA) followed by Tukey's comparison tests

(140 ± 3 s vs. control 180 ± 3.2 s, *P* <0.01). By administrating imipramine concomitantly with vitamin B6 on the last day, it caused a plunge in the immobility time (94 ± 14 s, *P* < 0.05 vs. vitamin B6 alone). Propranolol coadministration with vitamin B6 on the last day did not alter immobility time in comparison with vitamin B6 alone, whereas prazosin administered before the last dose of vitamin B6 further reduced the immobility time (89 ± 6 s, *P* < 0.01 vs. vitamin B6 alone). On the contrary, exposure to yohimbine on the last day of vitamin B6 therapy prevented the useful effects of vitamin B6 on the immobility time (150 ± 3 s). Administrating AMPT completely inhibited vitamin B6 effects on the immobility time (170 ± 8 s, *P* <0.05 vs. vitamin B6 alone) and the results were similar to the control amount.

The results of the mobile phase during FST showed that the swimming time [Figure 2B] increased when imipramine and prazosin were injected to vitamin B6–treated animals; 120 ± 12 and 130 ± 1 s, respectively (*P* < 0.01 vs. vitamin B6 alone). By observing [Figure 2C], it was found that vitamin B6 alone significantly increased climbing time (31 ± 2 s vs. 1.3 ± 1 s, control), neither of the drugs coadministered with vitamin B6 altered this figure except for yohimbine, which considerably reduced it to 11 ± 3 s (*P* < 0.05 vs. vitamin B6 alone).

Discussion

The following study showed that the antidepressant-like effects of vitamin B6 are influenced by the noradrenergic system modulatory drugs, which prove the possible involvement of the noradrenergic system in vitamin B6 antidepressant effects.

In FST, the immobility time is related to depressive behavior because it is changed by antidepressants and stimuli that provoke depressive behavior.[16] In addition, during the FST, more precise description of behavioral effects can be helpful

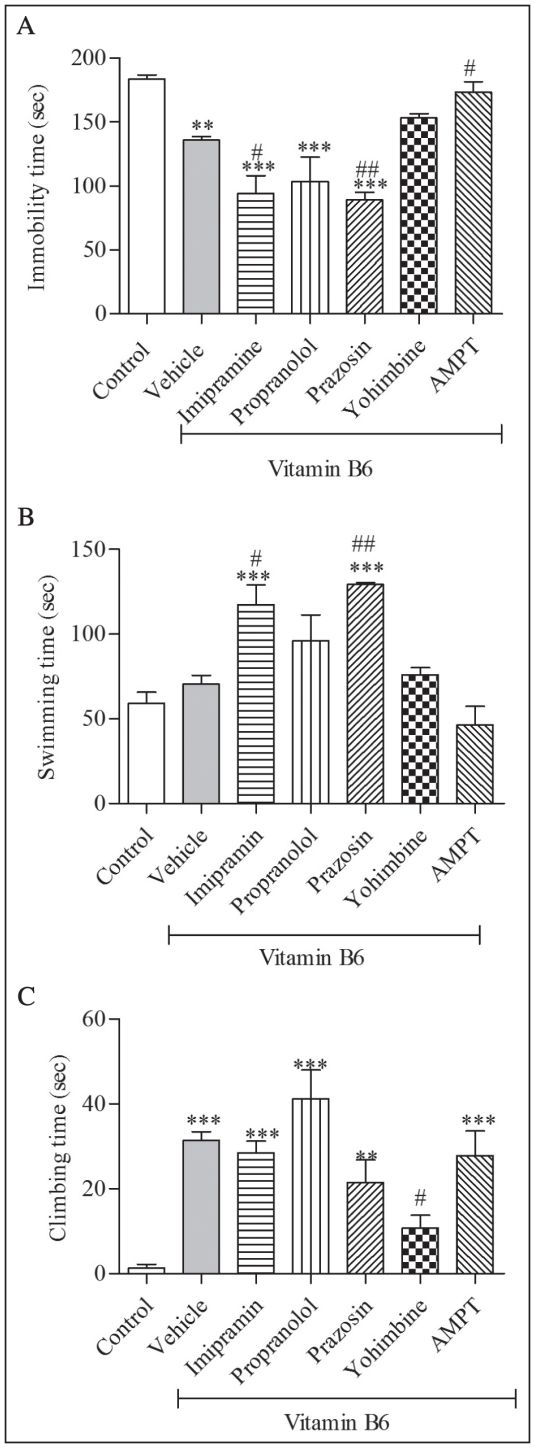

Figure 2: Effect of the drugs following vitamin B6 administration on behavior—(A) the immobility time, (B) the swimming time, and (C) the climbing time—in the forced swimming test. Vitamin B6 (100 mg/kg), imipramine (5 mg/kg), propranolol (2 mg/kg), prazosin (1 mg/kg), yohimbine (1 mg/kg), and α-methyl-*p*-tyrosine (AMPT) (100 mg/kg) were administered intraperitoneally. Number of animals in each group was six, control animals received normal saline. Vehicle group received normal saline + vitamin B6. Results are expressed as group mean ± standard error of mean and analyzed by analysis of variance followed by Tukey's *post hoc* tests ***P* < 0.01,**P* < 0.001 compared with the control group; #*P* < 0.05, ##*P* < 0.01 compared with the vehicle group**

in understanding the function of different neurotransmitter systems in the treatment of depression. The sequence of behaviors in the FST ordinarily appear like climbing that is performed during the first minutes of the test, periods of swimming occur throughout the interval, and immobility is most frequent at the end of the test. Climbing and swimming seem to be two separate responses that facilitate escape during the FST.[16] Following placement in an inescapable cylinder of water, the animals initiate escape-oriented movements, but they soon develop immobile posture. The immobility is reflected either as a progress of passive behavior that prevents the animal from active forms of managing the stressful condition or disappointment of persistence in escape behavior (i.e., behavioral despair).[17] Reference antidepressants reduce immobility time during the FST, whereas evaluating the mobile behavior may help to interpret different neurotransmitters. That is, the serotonin selective reuptake inhibitors (SSRIs) mostly increase swimming time, though climbing time is increased by antidepressant drugs with catecholamine transmission selective effects.[16,17]

The antidepressant-like effect of vitamin B6 that we observed during the FST is confirmed by previous data.[8] The following study extends earlier findings by providing convincing evidence that its effect during the FST is probably mediated by an interaction with the noradrenergic system and possibly α- and β-AR. The chosen doses of the adrenergic modulatory drugs (imipramine, propranolol, prazosin, yohimbine, and AMPT) when administered alone did not cause any noticeable change in the immobility time during the FST, which was in agreement with previous results [Figure 1].[13-15] In addition, they did not induce important change in animal activity during the locomotor test, which was also parallel with previous literature [Table 1].[18] Although their differences in the mobile phase (swimming and climbing time) during the FST reflects the variety of effect they might have caused on the neurotransmitters.

PLP is the cofactor for over 100 enzymatic reactions in the body, including the enzymes involved in the synthesis or catabolism of neurotransmitters (AADC synthesis of dopamine and serotonin). The role of NA in the pathophysiology of depression has been proved earlier.[2,19,20] Reduced noradrenergic system functioning is associated with depression,[19] and common antidepressants, such as TCAs, mainly act by increasing the synaptic availability of NA. Imipramine, a TCA, acts by increasing the activity of the brain's serotonergic or noradrenergic system by inhibiting the plasma membrane transporters for serotonin and NA.[21,22] Although the low imipramine dose alone did not change the immobility time in the FST, it induced profound antidepressant effects while coadministered with vitamin B6 [Figure 2A]. This reveals that vitamin B6 can improve imipramine antidepressant effect and it may induce similar effect on the neurotransmitter profile. Possibly the presynaptic and sometimes postsynaptic inhibitory α2-ARs combined with the excitatory postsynaptic

α1- and β-ARs play a role in the complex dynamics of NA modulation of target neurons.[23] This was verified by observing that the coadministration of propranolol (β-AR blocker) or prazosin (α1-AR blocker) following vitamin B6 therapy further reduced the immobility time, indicating that they augmented the antidepressant effect of vitamin B6, but yohimbine (α2-AR blocker) did not cause the similar change. In previous studies, the antidepressant effect elicited by lamotrigine, folic acid, or magnesium in the FST was reversed by prazosin and yohimbine.[14,15,18] Indeed, the α1- and α2-ARs have been proven to play a part in the antidepressant-like effects of drugs in behavioral models of depression.[15,24] Although the yohimbine results we observed concur with the previous findings, prazosin results are different. Evidently, chronic antidepressants and electroconvulsive therapy improve the density and functional activity of α1-ARs in the frontal cortex and hippocampus, and these receptors are linked to intracellular signaling pathways that control synaptic plasticity.[25] The α1B-AR subtype is essentially involved, which is in high density in corticolimbic structures and regions containing serotonergic and dopaminergic neurons.[25,26] In addition, a role of α1A-AR subtype has also been anticipated.[27] Therefore, the additional antidepressant effect of prazosin following vitamin B6 treatment, which was observed, opens new insights into the different effects of distinct α1-AR subtypes in depressive behavior. On the contrary, prazosin induced a rise in the swimming performance compared with vitamin B6 alone, which might be related to the serotonergic neurotransmitter.[16] The α1-AR stimulation in the dorsal raphe nucleus increases serotonin release,[28] which might be induced by prazosin and vitamin B6 interplay. Regarding the autoreceptors, α2A-AR subtype predominates over α2C-AR and its levels are higher in depressed individuals and following chronic stress.[26,29] Administrating yohimbine after vitamin B6 therapy slightly increased the immobility time or slightly reversed vitamin B6 antidepressant effects as no more difference was observed between this group and the control group [Figure 2]. Yohimbine also curtailed the climbing time in the FST, whereas the swimming time was unchanged when injected on the last day of vitamin B6 therapy. This further supports that vitamin B6 antidepressant benefits are related to the noradrenergic system. Literature is in favor of the beneficial effect of α2-AR antagonists on depression behavior following the administration of antidepressants,[30,31] but other researches are strongly against it.[32] Our results also supported the idea that the behavioral effect of antidepressants in the FST was antagonized by systemic administration of the α2-AR antagonist yohimbine.

The analogs of tyrosine, such as AMPT, are competitive inhibitors of tyrosine hydroxylase (the rate-limiting step in catecholamine synthesis) and they can induce depression in some individuals.[33] Depletion of the catecholamine system was specifically observed after treatment with AMPT.[23] Administration of AMPT following vitamin B6 therapy reversed the antidepressant-like benefits of vitamin B6 during FST. This further supports the involvement of the NA system in vitamin B6 antidepressant effect. The dose was chosen on the basis of previous research that showed treatment of mice with AMPT (100 mg/kg) was able to reverse the antidepressant effect of lamotrigine in the FST.[15] Thus, vitamin B6 cofactor effect on Dopa decarboxylase should be considered in its antidepressant effects observed in the FST. Thus, vitamin B6 by indirectly affecting the noradrenergic system by releasing NA induces antidepressant effects that may in turn interact with AR.

Conclusion

The results indicate that the antidepressant-like effect of vitamin B6 during the FST is mediated through an interaction with the noradrenergic system. The observations suggest that vitamin B6 enhances NA levels that in turn influences α1, α2, and β-AR. Nevertheless, monoamine neurotransmitters do not function individually, these neurotransmitter systems are naturally interconnected and alteration in one of these neurotransmitters likely affects the function of the others.

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Conflicts of interest

There are no conflicts of interest.

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