# Effects of calcium channel blockers on antidepressant action of Alprazolam and Imipramine

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**Abstract:** Alprazolam is effective as an anxiolytic and in the adjunct treatment of depression. In this study, the effects of calcium channel antagonists on the antidepressant action of alprazolam and imipramine were investigated. A forced swimming maze was used to study behavioral despair in albino mice. Mice were divided into nine groups (n = 7 per group). One group received a single dose of 1% Tween 80; two groups each received a single dose of the antidepressant alone (alprazolam or imipramine); two groups each received a single dose of the calcium channel blocker (nifedipine or verapamil); four groups each received a single dose of the calcium channel blocker followed by a single dose of the antidepressant (with same doses used for either in the previous four groups). Drug administration was performed concurrently on the nine groups. Our data confirmed the antidepressant action of alprazolam and imipramine. Both nifedipine and verapamil produced a significant antidepressant effect (delay the onset of immobility) when administered separately. Verapamil augmented the antidepressant effects of alprazolam and imipramine (additive antidepressant effect). This may be due to the possibility that verapamil might have antidepressant-like effect through different mechanism. Nifedipine and imipramine combined led to a delay in the onset of immobility greater than their single use but less than the sum of their independent administration. This may be due to the fact that nifedipine on its own might act as an antidepressant but blocks one imipramine mechanism that depends on L-type calcium channel activation. Combining nifedipine with alprazolam produced additional antidepressant effects, which indicates that they exert antidepressant effects through different mechanisms.

## Introduction

Alprazolam is an anxiolytic agent used primarily for short-term relief of mild to moderate anxiety and nervous tension. It is effective in the treatment of depression and panic attacks. It has a high affinity for the GABA benzodiazepine receptor complex [1], and it is a full agonist for the $GABA_A$ receptor [2]. Imipramine is a better tricyclic antidepressant than all other drugs in its category [3]. It prevents the reuptake of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) at nerve terminals [4].

Calcium antagonists have been shown to affect many different physiological processes, in particular neurotransmitter release. Nifedipine mainly affects the heart and smooth muscle, causing inhibition of calcium entry associated with depolarization. Nifedipine is relatively smooth muscle selective and acts as a vasodilator [1]. It is a highly specific antagonist of the L-type channel blocks [5]. Verapamil is relatively cardioselective with an antidysrhythmic action. Verapamil is effective in the treatment of hypertension and angina [1]. Verapamil enhanced the antidepressant action of alprazolam (6); Verapamil as an inhibitor of the CYP 450 3A4 (7) may affect the imipramine (8) and alprazolam action, that are considered as substrates for CYP 450 3A4 (7)

The forced swim test (FST) [9] is used as a rodent model of depression. The mouse FST model has been widely used in screening antidepressants because it is simple and has been reported to be reliable across laboratories. The mouse model is more sensitive than the rat model because it produces fewer false positives [10]. The FST is specific enough to discriminate between antidepressants, neuroleptics and anxiolytics [11]. It is based on the observation that when an animal is forced to swim in a situation from which there is no escape, it will first go through a period of vigorous activity and then cease to move, other than trying to keep its head above water. Immobility indicates a state of despair in which the mouse has learned that escape is impossible.

FST immobility is reduced by different treatments known to be effective in depression [12, 13]. There is a significant correlation between the potency of antidepressants in the FST and in clinical settings, but such a correlation has not been demonstrated in any other animal model of depression [12, 14]. In this model the circadian time cycle did not alter the duration of immobility of mice [15].

Behavioral despair is mediated by central catecholamines. Drugs that increase central transmission of dopamine or NA decrease immobility, whereas agents having the opposite effect increase immobility. The advantage of the mouse FST model is that it can readily test the possible mechanisms of antidepressant action by using specific agonists/antagonists. By augmenting or blocking antidepressant activity with agonist/antagonist receptor ligands, it is possible to detect which receptor is involved in the antidepressant effect [16].

In this study we used behavior despair models for mental depression to investigate the effect of the calcium channel blockers, nifedipine and verapamil, on the antidepressant action of alprazolam and imipramine. These two calcium channel blockers are used in the treatment of physical illnesses that may be concurrent with depression. Understanding the interaction between antidepressants and calcium channel

blockers could indicate whether there is a need to modify antidepressant doses when coadministered with calcium channel blockers.

## Materials and methods

Albino mice (25 - 40 g) were used. Groups of mice were housed in separate cages. The animals were housed at room temperature (20–25°C) and a 12-h dark/light cycle. Alprazolam was supplied by Upjohn Co. Ltd, Egypt, Imipramine by Novartis, AG, Switzerland, Nifedipine by Bayer, AG, Germany, and Verapamil by Weimer Phara Gmbh, Germany.

Because alprazolam is not freely soluble in saline, all the drugs were dissolved in 1% Tween 80 in distilled water [17]; they were injected intraperitoneally. Imipramine was given at 10 mg/kg and alprazolam at 5 mg/kg [18 and our unpublished results]. The doses of calcium channel blockers were chosen from previous studies [18-21].

Mice were divided into nine groups (n = 7 per group). One group received a single dose of 5ml/kg of 1% Tween 80; two groups each received a single dose of the antidepressant alone (alprazolam, 5mg/kg; imipramine, 10mg/kg); two groups each received a single dose of the calcium channel blocker (nifedipine, 5mg/kg; verapamil, 10mg/kg); four groups each received a single dose of the calcium channel blocker followed by a single dose of the antidepressant (with same doses used for either in the previous four groups). Drug administration was performed concurrently on the nine groups. For all groups, the time of onset of immobility was measured 60 min after drug administration.

We used a behavioral model of immobility first postulated by Porsolt [22] and Porsolt *et al.* and named the behavioral despair model [23]. In this model, mice are forced to swim in a restricted space from which there is no escape. Following an initial period of vigorous activity, the mice adopt a characteristic immobile posture with no further attempt to escape, and this reflects a state of despair or lowered mood. Mice were forced to swim for four minutes in a vertical glass cylinder (height=27 cm, diameter=16.5 cm) containing fresh tap water at 27°C and a depth of 15 cm [24]. Mice were forced to swim only once.

## Statistical analysis

SPSS 8 software was used to determine whether the observed data were normally distributed using Kolmogrov Smirnov maximum deviation test for goodness of fit. If the data were normally distributed, one-way ANOVA was applied, followed by *Post Hoc* test to compare between groups. If the data were not normally distributed, groups were compared using Mann-Whitney two samples (non–matched) *U* test. The difference was considered to be significant at $p \leq 0.05$.

## Results

### Effects of nifedipine on the onset of immobility

Administration of imipramine, alprazolam, or nifedipine separately produced a significant delay in the onset of immobility compared to the control group. The combined administration of alprazolam and nifedipine produced a significant delay in the onset of immobility compared to either alprazolam treated mice or the control group. The effect of imipramine on the onset of immobility (delay) was potentiated by the administration of nifedipine (Table 1).

Table 1: Effects of nifedipine on the onset of immobility by alprazolam and imipramine using the behavior despair model of depression. The values are expressed as mean ± S.E.M. a: $p \leq 0.05$ compared to control group treated with Tween 80-treated. b: $p \leq 0.05$ compared to group treated with alprazolam + nifedipine. c: $p \leq 0.05$ compared to group treated with imipramine + nifedipine.

| Treatment (n = 7) | Onset of immobility (sec) |
|---|---|
| Tween 80 | 40.5 ± 0.99 |
| Alprazolam (5 mg/kg) | 59.8 ± 1.16 a, b |
| Alprazolam (5mg/kg) + Nifedipine (5 mg/kg) | 72.1 ± 1.18 a |
| Imipramine (10 mg/kg) | 77.1 ± 0.83 a, c |
| Imipramine (10 mg/kg) + Nifedipine (5 mg/kg) | 83.4 ± 1.04 a |
| Nifedipine (5 mg/kg) | 60.1 ± 1.48 a, b, c |

### Effects of verapamil on the onset of immobility

Administration of verapamil, alprazolam or imipramine produced a significant delay in the onset of immobility compared to the control group. Coadministration of verapamil augmented the effects of imipramine. Similarly, coadministration of verapamil augmented the effects of alprazolam. Our findings demonstrate that verapamil significantly delays the onset of immobility produced by alprazolam (Table 2).

## Discussion

It has been suggested that calcium channel inhibitors may have antidepressant properties, and that calcium may play an important role in affective disorders [25]. Voltage-dependent calcium channel antagonists have been reported to produce antidepressant-like effects in rodents [26-28]. Interruption of the $Ca^{2+}$-calmodulin-NOS-guanylyl cyclase subcellular signaling pathway at any point produces antidepressant-like effects [26].

Table 2: Effects of verapamil on the onset of immobility produced by alprazolam or imipramine using the behavior despair model of depression. The values are expressed as mean ± S.E.M. a: $p \leq 0.05$ compared to control group treated with Tween 80-treated. b: $p \leq 0.05$ compared to group treated with alprazolam + verapamil. c: $p \leq 0.05$ compared to the group treated with imipramine + verapamil.

| Treatment (n = 7) | Onset of immobility (sec) |
|---|---|
| Tween 80 | 40.5 ± 0.99 |
| Alprazolam (5 mg/kg) | 59.8 ± 1.16 a, b |
| Alprazolam (5 mg/kg)+ Verapamil (10 mg/kg) | 82.8 ± 0.73 a |
| Imipramine (10 mg/kg) | 77.1 ± 0.83 a, c |
| Imipramine (10 mg/kg) +Verapamil (10 mg/kg) | 93.5 ± 1.13 a |
| Verapamil (10 mg/Kg) | 57.4 ± 0.61 a, b, c |

In our study, nifedipine delayed the onset of immobility in the forced swimming maze. This antidepressant action could have been mediated by 5-$HT_{1A}$ activation [29], whereby nifedipine reduced 5-HT uptake [30]. This would lead to an increase in the cytosolic calcium activity via 5-$HT_2$ receptors [31]. Serotonin may activate calcium influx through calcium channels by activation of 5-HT receptors, which are insensitive to nifedipine [32], in neuronal cells. The increase in calcium influx is through 5-$HT_3$ receptors [31, 33], the 5-$HT_3$ receptor being a ligand-gated ion channel activated by the neurotransmitter serotonin. Receptors of this subtype have been localized to several regions of the brain, they appear to be involved in many neuronal functions [34], and to mediate antidepressant effects [35]. In glial cells, the increase in intracellular calcium is through 5-$HT_2$ receptors [31]. It has been suggested that the pharmacology of L-type $Ca^{2+}$-channel blockers overlaps with that of 5-$HT_2$ receptor antagonists [36].

Nifedipine may produce an antidepressant action through GABAA activation [37], which leads to the release of NA that produces an antidepressant effect [38]. This may be GABA acting on second inhibitory interneurons (as in direct and indirect pathways of extrapyramidal systems). Nifedipine may also produce its antidepressant effect by increasing the release of intracellular calcium through GABAA receptors [37] and NA [39, 40]. The central antidepressant effect of nifedipine may be mediated through an interaction, with novel modulatory sites on GABAA receptors, that is not through picrotoxin, flumazenil or $Zn^{+2}$ sites [37].

Nifedipine may exert an antidepressant action by decreasing the ability of vesicles to re-uptake NA [41]. Nifedipine may block calcium uptake through potential operated ($K^+$) channels or through receptor operated (5-HT) channels [42].

Moreover, verapamil showed an antidepressant-like effect as it delayed the onset of immobility in the swimming maze. Studies have shown that verapamil modulates the action of antidepressant drugs that downregulate β adrenergic systems [43]. It has a similar final effect as β blockers, and shares this effect with antidepressant drugs [44]. Working on different types of calcium entry, verapamil blocks the prejunctional $\alpha_2$ receptors, which leads to an increase in NA release [45-48]. Verapamil may have direct catecholamine-releasing effects, as it interacts with catecholamine storage vesicles in a way that reduces their ability to take up and store catecholamine, and thereby increasing NA release from sympathetic nerves [41]. Verapamil has no effect on NA-induced increase in calcium influx, which means that there are verapamil-sensitive and verapamil-insensitive calcium channels [49]. Verapamil enhances ATP response [50], which is released along with NA from the motor nerves; ATP may indeed be a co-transmitter [51, 52]. Allgaier *et al.* suggested that ATP induces NA release from sympathetic neurons via its action on a subclass of the nicotinic cholinoceptor, because this effect was blocked by nicotinic receptor antagonists [53]. NA produces depolarization by decreasing the membrane permeability to $K^+$ ions. It also increases calcium influx to the cells via calcium channel-activated NA receptors and potential-dependent slow calcium-channels activated by NA-induced membrane depolarization [54]. NA stimulates calcium chloride conductance, leading to opening of voltage-gated calcium channels [55].

Verapamil inhibits 5-HT uptake by a mechanism that does not involve alteration in calcium fluxes (56), which leads to enhancement of 5-HT release

[57]; verapamil produces competitive inhibition of a 5-HT carrier and inhibits $Na^+$-dependent uptake of 5-HT [58, 59], which is competitively inhibited by imipramine [59]. It also facilitates the release of large amounts of $K^+$-induced 5-HT in the hippocampus synaptosomatic sites. The release is dose dependent and mediated by presynaptic receptors. This increase in endogenous 5-HT is independent of the presence of external calcium [60]. Serotonin activates $5\text{-HT}_{1A}$ receptors to produce an antidepressant effect [61]. It also activates $5\text{-HT}_3$ presynaptic receptors, inducing calcium influx [62, 63], which triggers the release of calcium from intracellular stores and leads to increased calcium in both the cytoplasm and nucleus [63]. Activating $5\text{-HT}_3$ postsynaptic receptors induces depolarization [62]. Thus both mechanisms would result in an antidepressant action.

Serotonin may regulate GABA neurotransmission through $5\text{-HT}_3$ receptors [64]. It also increases chloride channel activity by acting on the $5\text{-HT}_{1C}$ receptor [65, 66]. It has been suggested that the increased $[Ca^{2+}]_i$ activates chloride channels (Cl), causing an efflux of chloride and subsequent depolarization of the cell membrane; this leads to the opening of voltage-gated calcium channels [67,68]. $Cl^-$ currents can be activated separately by $Ca^{2+}$ release from intracellular stores (in response to external application of caffeine or NA) and by $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels [69]. Activation of Cl- channels by $Ca^{2+}$ release produces a membrane depolarization that is required for an enhanced opening of voltage-dependent $Ca^{2+}$ channels in response to noradrenaline in venous smooth muscle [70]. It was concluded that extracellular chloride is essential for contraction in afferent arterioles after activation of voltage-dependent calcium channels [71].

Verapamil cannot modify calcium-dependent fractions of GABA release induced by high $K^+$ depolarization [72]. Presynaptic action of Zn in the hippocampus increases the release of GABA in the synaptic cleft. This Zn effect is not affected by verapamil. Zinc enhances GABA release by potentiating AMPA/Kaiate receptors in the hippocampus region, followed by a decrease in presynaptic glutamate release. Zinc is considered an inhibitory neuromodulator of glutamate [73]. The NMDA receptor gates two inhibitory sites for zinc, one is voltage dependent (74) and the other is not (74-76). The γ-amino butyric acid released from striatal slices is calcium-independent. Calcium channels may play two roles in the regulation of depolarization-induced GABA release. First, they permit depolarization-induced influx of calcium, which then promotes GABA release. Second, they influence GABA release through a mechanism that does not involve external calcium. It has been proposed that calcium channels serve to permit an influx of $Na^+$, which in turn promotes calcium-independent GABA release through an influence on the high affinity GABA transport system [77]. Blockage of choline uptake may play a role in the antidepressant-like effect of verapamil [78].

Alprazolam may produce antidepressant effects independently of benzodiazepine receptors, through the GABA–ergic mechanism. Flumazenil did not antagonize the antidepressant effects of alprazolam or imipramine, while these effects were blocked by picrotoxin [79]. Enhanced 5-HT release in the hippocampus, exhibited by the atypical benzodiazepine, alprazolam, but not by the typical benzodiazepine, diazepam, may underlie the antidepressant activity of alprazolam [80]. It has been suggested that some of the neuronal stabilizing effects of benzodiazepine receptors may be mediated by the regulation of $Ca^{2+}$ conductance, as benzodiazepine binding sites regulate voltage-sensitive $Ca^{2+}$ channels in brain membranes [81]. Benzodiazepines and calcium channel inhibitors cause significant inhibition of adenosine transport; hence, this potentiates adenosine action at the concentration required to induce effects through occupation of their respective, specific high-affinity sites [82]. Benzodiazepine (clonazepam, flunitrazepam, and diazepam) binding sites regulate voltage-sensitive $Ca^{2+}$ channels in brain membranes, and it has been suggested that some of the neuronal stabilizing effects of benzodiazepine receptors may be mediated by the regulation of $Ca^{2+}$ conductance [81]. Diazepam had no effect on the immobility time in the swimming maze, but antagonized the antidepressant effect of imipramine [83]. This means that both alprazolam and imipramine produce antidepressant effects through voltage-sensitive $Ca^{2+}$ channels. Benzodiazepines act as $Ca^{2+}$-channel antagonists [84]; this antagonism may not be related to their antidepressant action [85, 86].

Imipramine can inhibit presynaptic reuptake of the biogenic amines, serotonin, and NA to produce an antidepressant action [87-89]. Imipramine may produce this antidepressant action through a GABA–ergic mechanism, causing release of catecholamines [79]. Imipramine may increase calcium release from intracellular stores [90] by increasing NA concentrations through inhibiting its uptake by pre-synaptic sites [87], through GABAA receptor activation. This may

lead to increased calcium influx through voltage-gated calcium channels, which ultimately depend on the chloride transport system [91], or by depolarization due to an increase in the external potassium concentrations. It was suspected that this might lead to calcium influx through voltage activated calcium channels [92]. However, nifedipine does not affect calcium channel mediation of initial response to NA [93]. Nifedipine blocks L-type calcium channels activation which is due GABAA receptor activation-mediated depolarization [94, 95], which may not play a role in the antidepressant action. GABAA receptor activation increases the release of calcium from the internal stores [95]. Imipramine produces an inhibition of the peak threshold calcium current, which probably decreases the maximum available calcium conductance [96]. It was suggested that imipramine acts by interfering with the influx of extracellular calcium, through both the receptor-operated and voltage-gated calcium channels, but does not affect the release of calcium from intracellular storage sites [97, 98].

In the present study we showed that verapamil has an antidepressant-like effect in the mouse swimming maze model. Treatment with verapamil combined with alprazolam or imipramine produces an additive antidepressant effect, possibly because verapamil has an antidepressant-like effect, but the mechanism is not understood yet.

Either imipramine or nifedipine produced a delay in the onset of immobility of 75% and 81%, respectively, compared to the control. Combining nifedipine with imipramine led to a delay of 73% in the onset of immobility compared to the control; which is less than the additive effect. This observation could be explained by the fact that nifedipine has its own antidepressant action mechanism but also blocks the imipramine mechanism that depends on L-type calcium channel activation.

This study showed that nifedipine possesses antidepressant properties. Combining nifedipine with alprazolam produced an additive antidepressant effect, indicating that different mechanisms were involved.

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