Effect of nifedipine on alprazolam-induced anxiolysis and brain GABA level changes in albino rates

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ABSTRACT

Objectives: The present study investigates the effects of alprazolam (ALP) and nifedipine alone or in combination on behavior and on -aminobutyric acid (GABA) levels, in discrete brain regions of albino rats.

Methods: The anxiolytic effect was studied using a plus maze model and brain levels of GABA were measured using high performance liquid chromatography. Four acute treatment groups of rats were used. In the first they were treated with 1% Tween 80 (1ml/kg), in the second with nifedipine (10mg/kg), in the third with ALP (2mg/kg) and in the fourth with ALP in addition to nifedipine in the respective doses. The work was carried out at the Faculty of Pharmacy of Al-Fateh University, Tripoli, Libya in the first half of 2002.

Results: The results indicate that the anxiolytic effect of ALP was not modified by nifedipine. Nifedipine by itself significantly decreased the motor activity (decrease in total lines crossed), this effect was apparently antagonized by ALP. Alprazolam administration produced an increase of GABA levels in cerebellum and striatum and a decrease in the brain stem. Nifedipine per-se had no effect on GABA levels in the brain stem but it partially antagonized ALP-induced inhibitory effect on GABA in this region. Alprazolam significantly increased GABA levels in the striatum, while nifedipine alone had no effect on neurotransmitter levels and did not modify the ALP effect in this brain region. Alprazolam or nifedipine had no significant effect on GABA levels in midbrain, cerebral cortex and whole brain. There were no significant changes in GABA levels in midbrain and whole brain with drug combination. However, the combination decreased GABA levels significantly in the cerebral cortex.

Conclusion: It may be concluded that, the anxiolytic effect of ALP possibly occurs through changes in brain GABA levels (an increase in cerebellum and striatum with a decrease in brain stem). The effect was not modified by nifedipine which per se had no affect on GABA levels in any brain area. The significant decrease in GABA levels in cerebral cortex by ALP-nifedipine combination may be due to the mutual closure of calcium channel (mentioned in literature) resulting in inhibition of the EAA-ergic input to GABA-ergic neuron.

Neurosciences 2003; Vol. 8 (2): 96-103

The prominent effects of benzodiazepines - sedation, hypnosis, decreased anxiety, muscle relaxation, anterograde amnesia and anticonvulsant activity, virtually all result from actions of these drugs on the central nervous system (CNS). Alprazolam (ALP), one of the benzodiazepines, also has antidepressant activity in a clinical study.[1-3] The drug is an anti-anxiety agent, used primarily for short-term relief of mild to moderate anxiety.[4-7] It is also effective in the treatment of depression or in panic disorders with or without agoraphobia.[7] The benzodiazepine receptor (BZR) is an allosteric modulatory site within the -aminobutyric acid (GABA)A receptor-chloride channel complex. The nature and distribution of GABA-BZR complex is shown to be essentially similar to human and rat brain.[8,9] Agonists and partial agonists enhance apparent GABA

potency at GABA$_A$ receptors maximally (2-3 fold) or submaximally.[10] It is possible that the dichotomy of the antidepressant effects of ALP[11,12] from the anxiolytic effects may be related to the degree of receptor occupancy or the pharmacokinetic difference amongst 1,4-benzodiazepines.[13,14] The major molecular target for BZ is the inhibitory neurotransmitter (GABA) system[15,16] which affects nearly all of the CNS[17,18] and produces anti-anxiety action.[19,20] Furthermore GABA-mimetics such as muscimol, 4,5,6,7-tetrahydroisoxazolo [4,5-c]pyridine-3-ol (THIP), isoguvacine and aminoacetic acid have been shown to have anti-anxiety effects in social interaction tests[21-23] and in the elevated-plus maze.[24] Alprazolam binds with high affinity to the GABA benzodiazepine receptor complex and its central pharmacological/therapeutic actions are mediated via interaction with this receptor complex.[7] It is well known that GABA receptor stimulation produces opening of chloride (Cl$^-$) channels resulting in hyperpolarization.[25] However GABA is also related to calcium channels as evident from a recent study which mentions that astrocytes accelerate the developmental change in the Cl$^-$ ion gradient extrinsic to GABAA receptor/Cl$^-$ channel, which is critical for triggering calcium (Ca^{2+}) entry, without influencing parallel changes in the intrinsic properties of the channels.[26] It was also found that GABA response shares a common pathway of Ca^{2+} movement with the high potassium (K$^+$) induced response; suggesting that the stimulation with GABA results in Ca^{2+} influx through voltage-gated Ca^{2+} channels, and these effects are dependent on Cl$^-$ transport systems.[27] Furthermore it is substantiated by the study which shows the ability of progesterone, GABA, or muscimol to stimulate acrosomal exocytosis in mouse spermatozoa which was blocked by the Ca^{2+} channel antagonist - nifedipine[28] which, like most channel blockers prevents the opening of voltage gated Ca^{2+} channels [Long lasting (L) and transient (T) types].[25] It has recently been shown that GABA or muscimol have a depolarizing effect on Ca^{2+} channel which is antagonized by nifedipine.[29] It has been explained that the mechanism by which GABA receptor stimulation results in elevated intracellular calcium [Ca^{2+}]i, is due to membrane depolarization by increased Cl$^-$ conductance resulting in calcium influx through L-type voltage-dependent calcium channel in neonatal rat pituitary cells.[30] The experimental work on the effect of ALP on animal behavior is scanty.[31,32] Most of the studies concentrated on the swimming maze model for ALP antidepressant action.[33-36] Clinically, ALP (xanax) may be prescribed with calcium channel blockers which are used as antihypertensive (nifedipine and diltiazem) and anti-angina (nifedipine and diltiazem) remedies. With these relationships of ALP modifying the GABA-receptor complex, the interaction of GABA-receptor with calcium channel and the possibility of concomitant use of ALP with Ca^{+2} channel blockers, the present work was undertaken to evaluate the effect of ALP, as an anxiolytic drug, its effect on GABA levels in different brain regions and possible interaction on behavior and GABA levels when both are administered together.

Methods. -amino-n-valeric acid (valine; 5-aminopentanoic acid); -amino-n-butyric acid (GABA; 4-aminobutanoic acid; Piperidic acid) were obtained from Sigma Chemical Company, United States of America (USA); acetonitrile and methanol chromasol for liquid chromatography from SDS, France; phosphoric acid was obtained from Riedel-De Haen AG Seelze Hanover, Germany; perchloric acid from May & Baker Ltd, England; potassium carbonate and sodium hydrogen carbonate from BDH Limited, England; dansyle chloride (5-dimethylamino naphthalene-1-sulfonyl chloride), anhydrous acetone from Koch-Light Ltd, England, glacial acetic acid from BDH Limited, England and potassium dihydrogen phosphate was obtained from Riedel-De Haen AG, Germany. Alprazolam (ALP) was supplied by Upjohn Company, Egypt; and Tween 80 (T80) from Riedel-De Haen AG, Germany. Nifedipine was supplied by Bayer Company, Germany.

Male albino Wistar rats weighing between 150 and 250 gms were used. Each group was housed separately in a cage, except during the time of measurements. Standard rat food pellet diet and water were available ad lib. The animals were kept at constant room temperature (20-25°C), and 12 hours dark/light cycle. Alprazolam and nifedipine were administered by the intraperitoneal route. A volume of injection of 1.0ml/kg of body weight was adopted.[37] The suspending agent used for ALP was 1%Tween 80 in water.[38]

Acute administration. The rats were divided into 4 equal groups of 6 animals each. Group I the control group received only a single dose of 1% Tween 80 (T80). Group II received a single dose of 2mg/kg ALP. Group III received a single dose of 10mg/kg nifedipine. Group IV received doses of 2mg/kg ALP and 10mg/kg of nifedipine at the same time. Behavioral measurements using elevated plus maze were scored immediately after 30 minutes of drugs administration.[39-41] The levels of GABA in brain were measured at the end of the behavior measurements.

Behavioral measurements. The elevated plus-maze was made of wood with 2 opposite open arms (50 x 10cm) and 2 opposite closed arms of the same size with walls of 40cm height. The arms were connected by a central square (10 x 10cm), thus the maze formed a plus sign. The plus-maze was elevated 50cm above the floor. The plus-maze test was conducted between 9am and 1pm in a closed room with low level of illumination[42] and under constant conditions of temperature, humidity and the natural light-dark cycle. Each rat was placed on the central square of the plus-maze, facing one of the closed arms, and was observed for 4 minutes of free exploration. The numbers of entries into arms, lines crossed and times spent on open and closed arms were

scored. An entry was defined as having both forepaws in the respective arm. Line crossing was defined as both forepaws crossing the line.[43] Anxiety measure (AM) was calculated by dividing the time spent in closed arm by the total time of the test.

Measurements of GABA. Rats were killed by cervical dislocation and decapitation, and the body was exposed to a microwave irradiation for 4 seconds.[44-46] The brains were quickly removed and placed on an ice-cooled glass plate. The cerebellum was removed first by cutting the cerebellar peduncles, followed by the pons and medulla which were separated from the rest of the brain. The cerebral cortex was then pulled away to expose the corpus callosum to expose the lateral ventricles. The striata protruding from the inner surface of the cerebral hemispheres into the ventricles together with the caudate nuclei, were removed by gently scraping the inside of the ventricle with a sharp scalpel. The cerebral hemispheres were then dissected from the rest of the brain.[47,48] The removed brain regions were weighed and were placed in 100 ml plastic tubes previously placed in iced bath and containing 10ml of ice-cooled 0.1M perchloric acid.[44,45] The tissues were homogenized in Fisher Dyna-Mixer (Fisher Scientific, USA) for one minute keeping the tube embedded in an ice bath, and then centrifuged at 5000 rpm for 10 minutes at 4°C. The supernatants were stored at 20°C until assayed. Calibration curves were constructed by carrying solutions of standard GABA (25, 50, 100, 200 and 300 μg/ml) in 1.0ml perchloric acid, each containing 150μg/ml of valine as an internal standard. One milliliter of each standard solution was diluted to 10ml with perchloric acid. Dansylation was carried out by taking 100μl of the supernatant of the samples or the standards and added to a micro-tube containing 100μl of 0.1M potassium carbonate solution. The solutions were mixed using vortex mixer and then centrifuged using microcentrifuge at 10.000 rpm for 10 minutes. A 100μl of the supernatant of the centrifuged mixture was transferred into a pyrex tube containing 100μl of 0.1M sodium hydrogen carbonate solution, and then adding 400μl of working dansyl chloride solution. The tubes were shaken for 30 seconds using vortex mixer and then incubated at 90°C in benchtop oven for 30 minutes. The tubes were not capped during the incubations, so as to allow much of the sample to evaporate during incubation. This did not appear to adversely affect the progress of the dansylation reaction and served to concentrate the samples. After removal from the oven, the tubes were left to cool down to room temperature, and the dansylated derivatives were transferred into 1.5ml microtubes and stored at 20°C until assayed.[46] The high performance liquid chromatography (HPLC) system used to resolve and quantify the samples,[45] consisted of an LKB system from LKB-Produkter AB, Bromma, Sweden. It is formed of solvent conditioner (2156 LKB Bromma), column oven (2155 LKB Bromma), pump (2150 LKB Bromma), and recording integrator (2220 LKB Bromma). The HPLC columns were 5mm, 250 x 3.2mm, C8 reversed -phase column

from Phenomenex, St. Torrance, CA90501 USA. The flourometric detector was made by Phillips (Japan). The HPLC mobile phase[46,48] consisted of helium degassed deionized water-acetonitrile (HPLC grade) mixture (65:35, v/v) containing 0.15% (v/v) phosphoric acid. The flow rate was kept at 0.5 ml/min. The detector excitation was at 333nm and emission at 532nm. Twenty-five microliters of the dansyl derivative of GABA sample was transferred to HPLC microsample vials and injected into the column. The peak ratios of the samples were calculated with reference to the internal standard. The concentration of the samples was calculated from concentration-peak ratio curve of dansylated standards. Linear regression and sample concentration was calculated using windows 3.1 (Excel) software package. Retention time of GABA and internal standard were found to be in the range of 4.96, and 5.85 minutes.

Statistical analysis. Descriptive statistical analysis was applied on parameters of different samples using Statistical Package for Social Sciences version 8 to find out whether the observed samples were normally distributed using Kolmogorov-Smirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, treatments were compared in pair-wise fashion by applying one way ANOVA. If the parameters were not normally distributed, the treatments were compared by applying the Mann-Whitney 2 samples (non-matched) test. The differences were considered to be significant at P<0.05.

The work was carried out at the Faculty of Pharmacy of Al-Fateh University, Tripoli, Libya in the first half of 2002.

Results. The behavioral results using the plus maze are shown in **Table 1**. Acute administration of ALP produced a significant increase in time spent in open and zero areas, compared to the effect of acute 1% Tween 80 (control group). Alprazolam also significantly decreased the time spent in closed areas. Administration of nifedipine significantly antagonized the effect of ALP in open areas, and insignificantly in closed and zero areas. The anxiety measure was decreased significantly by ALP compared to control (T80 treated group), this decrease was insignificantly antagonized by nifedipine although the anxiety measure remained less significantly than the control. The numbers of entries into the open areas increased after the administration of ALP; this effect was abolished by nifedipine administration when compared to the control. The number of entries into the closed areas and the total number of entries (into the closed and the open areas) were not changed by any of the different treatments. The effect of acute administration of drugs (ALP and nifedipine) on spontaneous motor activity was studied with respect to the total number of lines crossed in all areas and in different individual areas. Alprazolam neither changed the number of lines crossed in closed areas nor the total lines crossed. Nifedipine however

showed significant decrease in the total lines crossed; the effect was abolished by ALP compared to the control. The results and the statistical analysis for the changes in GABA concentrations in different brain areas are given in **Table 2**. The acute administration of ALP (2mg/kg) increased the levels of GABA significantly in the cerebellum. Nifedipine per se had no effect on cerebellum GABA levels and it also failed to antagonize alprazolam effect in this region. In brain stem, ALP decreased GABA levels significantly. Commitment administration of nifedipine apparently abolished the inhibitory effect of ALP. Nifedipine administration alone had no effect on GABA levels. The levels of GABA were highly increased in striatum after administration of ALP; this increase was not affected by nifedipine when administered with ALP. Nifedipine by itself had no effect on GABA levels in striatum. Alprazolam had no significant effect on midbrain GABA levels. Nifedipine neither had any effect on GABA levels nor it modified the effect of ALP. In cerebral cortex, both nifedipine and ALP administered alone had no effect on GABA levels, but in combination, GABA levels were significantly decreased compared to the control. In the whole brain, there was no change in GABA levels by any of the treatments.

Discussion. Using plus maze model, acute ALP administration produced a significant decrease in anxiety measure (**Table 1**). Alprazolam treated rats spent more time in open and zero areas, less in closed areas and increased the number of entries into open arms, compared to the control (T80 treated groups). It should be mentioned that time spent and number of entries into open arms are negatively related to anxiety, while the time spent on the closed arms is positively related.[49] This agrees well with our results. The anxiolytic action of BZ is due to the binding to benzodiazepine receptor subtype1 (BZR1) which results in the anxiolytic non-sedative action[50] activating BZ-GABA-chloride channel complex.[51,52] Different parameters were reported in the literature to measure anxiolytic effect and motor activity in the elevated plus maze. These parameters include: lines crossed in open, closed and in all areas; time spent in open, closed and in all areas; number of entries into open, closed and into all areas. The relationship of these parameters to anxiety and motor activity was studied by Cruz and his colleagues[49] in rats left on elevated plus maze for 5 minutes. They mentioned 2 independent factors emerged from a factor analysis. The variables that loaded highly and negatively on factor one (seemingly related with anxiety) were: number of entries and time spent in open areas, percentage of open/total areas entries and percentage of time on open areas/total time of the test. The time spent on closed areas loaded highly, but positively on factor one. On the other hand, number of the closed arms entries, total number of arms entries and rearing loaded highly on factor 2 (probably related to motor activity). However, the total number of entries also loaded on factor one, being thus a mixed index. Similarly, the number of open arm entries loaded on both factors one and 2. Based on this, it is concluded that, ALP has an anxiolytic action (increase time spent in open and zero areas; decrease time spent in closed areas - lines and number of entries into open areas was increased) without any sedative effect (total lines crossed and total entries was not changed).

Nifedipine alone in the dose used (10mg/kg) did not change the time spent in any areas. Most of the known calcium antagonists act preferentially or solely on the L channel[53,54] which has no significant depressant effects on CNS.[55] However, it is known that the calcium entry that triggers neurotransmitters release through the N channels.[25] Nifedipine did not change the anxiolytic effect of ALP, since it has no effect on the calcium entry mechanism that triggers neurotransmitter release related to anxiolytic action. Nifedipine by itself decreased the motor activity (decreased the total lines crossed), this may be due to block of calcium channel at motor neurons which may produce muscle relaxation (unpublished results), leading to decreased motor activity (**Table 1**). Nifedipine-induced decrease in motor activity was apparently antagonized by ALP; this may be due to the anxiolytic action that may increase the animal exploratory behavior, resulting in increase in lines crossed.[56,57] It is therefore concluded that ALP produces anxiolysis and this action is not changed by the administration of nifedipine.

In the present investigation, an attempt has been made to correlate behavioral effects of drugs with brain GABA level. Acute administration of ALP produced significant increase in GABA levels in cerebellum and striatum (**Table 2**). This increase might be due to increase GABA synthesis through the stimulation of glutamate decarboxylation.[58] Thus the therapeutic effects of BZ (sedation, hypnosis or antianxiety) might be due to the increase in GABA levels in the brain regions (cerebellum, cerebral cortex, the limbic system and reticular formation) where the BZ binding sites are present.[59] The main brain regions for the presence of BZ1 receptor subtype are cerebellum, inferior colliculus, globus pallidus and substantia nigra pars reticularis.[60] This facet of BZ action may be one apart from the well known mechanism through the full selective allosteric modulation maximizing GABA action.[61,62] The modulator allosteric site on GABA-chloride channel complex mediates both facilitatory (BZ-receptor agonists) and inhibitory (inverse agonists) effects.[63] The other mechanisms that may contribute to anxiolytic effect involve neither GABA nor alterations in membrane permeability to chloride ion, and include inhibition of uptake of adenosine and potentiation of endogenous neuronal depressant adenosine,[64] GABA-independent inhibition of Ca^{+2} current,[65,66] Ca^{2+}-dependent release of neurotransmitters[25] and inhibition of tetrodotoxin sensitive Na^+ channel.[67] It is also possible that the action of ALP on GABA may be through the endogenous neurosteroids since it has been

Table 1 - Effect of alprazolam alone and in combination with nifedipine on behavior using the plus maze.

Treatments	Time spent in different areas				Number of entries into different areas			Lines crossed in different areas		
	Open	Closed	Zero	AM	Open	Closed	Total	Open	Closed	Total
1%T80 (1ml/kg)	0.0	239.17±0.307	0.833±0.307	0.997±0.0012	0.0	1.17±0.166	1.17±0.166	0.0	9.33±1.173	**9.33±1.173**
Nifedipine (10mg/kg)	2.57±2.57	212.71±14.54	24.71±12.546	0.886±0.061	0.143±0.143	1.29±0.285	1.43±0.429	0.285±0.285	5.43±1.586 *	**5.71±1.847** *
Alprazolam (2mg/kg)	43.5±38.97 *	146.83±37.45 *	49.67±25.914 *	0.612±0.156 *	0.67±0.333 *	1.83±0.703	2.5±0.847	1.16±0.654 *	9.50±4.794	**10.66±5.064**
Alprazolam +nifedipine	24±24	196.83±24.69 *	19.02±8.592 *	0.820±0.103 *	1.0±1.0	2.33±0.714	3.33±1.476	4.16±4.16	11.17±4.672	**15.33±7.783**

The values are the means ± SE for 6 rats
AM - Anxiety measures (time spent in closed areas/total of the test)
*Significantly different from T80 treated group at $p<0.05$

Table 2 - Effect of alprazolam alone and in combination with nifedipine on GABA levels in different brain areas.

Treatments	Cerebellum	Brain stem	Striatum	Midbrain	Cerebral cortex	Whole brain
1%T80 (1ml/kg)	184.44±10.741	170.85±10.259	405.93±37.830	245.40±8.499	174.85±8.906	212.56±16.46
Nifedipine (10mg/kg)	202.02±3.549	148.33±7.374	393.96±25.087	248.45±11.794	169.26±9.194	227.65±10.105
Alprazolam (2mg/kg)	284.98±28.473 *	127.35±10.424 *	996.63±57.228 *	285.17±22.436	152.89±19.702	221.29±10.027
Alprazolam +nifedipine	262.86±10.177 *,†	158.79±7.364	831.62±82.065 *,†	278.70±21.525	134.66±9.378 *,†	215.90±12.312

The values are the means ± SE for 6 rates
* Significantly different from the T80 treated group (control) at $p<0.05$
† Significantly different from nifedipine treated group at $p<0.05$
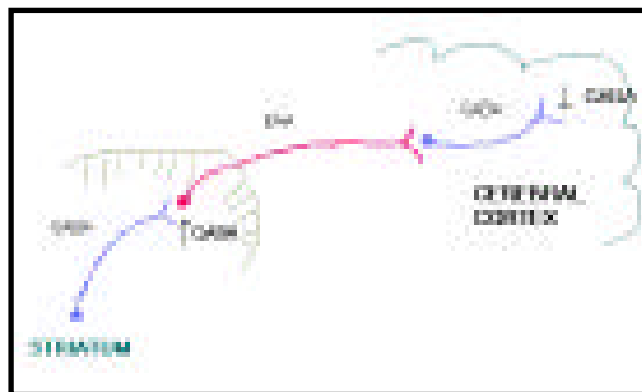

Figure 1 - GABA - EAA interrelationship in the central nervous system. GABA - -aminobutyric acid. EAA- excitatory amino acid.

shown that, mitochondrial and glial BZ receptors[68] increase neurosteroidal biosynthesis. Furthermore, the neurosteroids stimulate GABA and its neurotransmitter mechanisms[69-71] and may be related to anxiolysis.[72-74]

The increase in GABA levels by ALP was observed only in cerebellum and striatum, probably ALP dose may not be enough to produce action in other areas. Cerebellum is known to be involved in emotional cognitive processes and anxiolysis.[75] On the other hand, striatum has a key position to serve as an integrating unit modulating cerebral cortex, thalamus and the limbic system functions.[76-78] Striatum is previously shown to have maximum GABA level increase by low doses of triazolam[48] and it is also very sensitive to acute stress where GABA levels, in rat corpus striatum, were reduced by acute immobilization stress.[79]

Administration of ALP alone produced a significant decrease in brain stem GABA levels. It may interact with a low affinity site that results in negative allosteric action thus inhibiting GABA currents.[80,81] This effect may be through direct inhibition of the influx of extracellular Ca^{+2},[82] or reduction of the transient currents through type one calcium channel.[65] Nifedipine administration by itself had no effect on GABA levels in any of the brain areas, although it showed an insignificant decrease in brain stem. It is possible that intracellular calcium entry through nifedipine-sensitive calcium channels may have no effect on GABA release. Alternatively nifedipine dose may not be enough to produce any changes in brain GABA levels.

Calcium plays an important role in post synaptic membrane function and in neurotransmitter secretion from presynaptic terminals.[83] The calcium channels in CNS have different structural and functional characteristics.[84] It is becoming increasingly apparent that multiple receptors systems of different neurotransmitters in brain produce their responses by indirectly altering calcium channel activity (for example, altering membrane potential or threshold) without directly affecting the channel itself.[85] Voltage sensitive calcium channels have different protein structures which provide considerable latitude for the development of drugs with specific effects on these calcium channel receptor subtypes in the CNS.[85] Nifedipine in this study failed to antagonize ALP effect on GABA in cerebellum and striatum. Therefore it is possible that nifedipine exclusively acting on L-type calcium channel and not on N-type which is responsible for neurotransmitter release.[25,55] In cerebellum and striatum, GABA synthesis or secretion, may be controlled by calcium released from the sarcoplasmic reticulum[86,87] and not affecting calcium entry through the channel which is not antagonized by calcium antagonists.[25] The dihydropyridines affect Ca^{+2} channel function in a complex way, not simply by a physical plugging of the pore but binding to the same site acting in the converse way, namely promoting the opening of voltage-gated calcium channels.[88] Nifedipine apparently abolished the ALP-induced decrease in GABA levels in brain stem. Nifedipine may act in converse way (vide supra); the entry of extracellular Ca^{+2} can trigger the release of additional Ca^{+2} from the intracellular stores[55] which may lead to the release of neurotransmitter (GABA), as a result of this mechanism nifedipine may antagonize the ALP induced decrease in GABA levels in brain stem.

Benzodiazepines are shown to increase GABA levels in cerebral cortex.[48] However in the present investigation ALP did not produce any significant changes. Nifedipine also did not change GABA levels in cerebral cortex. However the administration of nifedipine and ALP together produced a decrease in GABA levels. It is possible that ALP-induced increase in striatal GABA levels cause inhibition of excitatory neurons going to the cerebral cortex.[89] This effect may be through blocking[90] or reducing the transient (T-type or type I) and long lasting (L-type or type II) type of calcium channel currents.[65] Nifedipine when given with ALP, there will be summation of effects on calcium channel causing its closure (**Figure 1**).

Therefore it may concluded that ALP in the dose used produced anxiolytic non-sedative action. It increased GABA levels in cerebellum and striatum which may be related to the anxiolytic effect. Alprazolam decreased GABA levels in brain stem, which was abolished by nifedipine. Nifedipine alone had no effect on anxiety measure, but decreased motor activity which was abolished by ALP. Nifedipine alone did not change GABA in cerebral cortex, but combination with alprazolam produced a significant decrease in GABA levels. It seems that calcium channel is complexly related to GABA in different brain regions in different directions.

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