Ann Natl Acad Med Sci (India), **43(2):** 23-34, 2007

Revisiting Gaba Receptor Pharmacology

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Summary

γ-Amino butyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. Almost 60-75 % of the neurons in the central nervous system are GABAergic in nature and they are virtually absent outside the brain and the spinal cord. GABA produces inhibitory hyperpolarizing response by increasing the chloride (Cl^-) conductance of the neuronal membrane. Increased GABAergic activity produces CNS depression, sedation, ataxia and amnesia. Any loss in GABAergic tone would lead to arousal response, insomnia and excitation. GABA is known to act on three distinct receptor subtypes, of which $GABA_A$ receptors are widely studied. The $GABA_A$ receptor is a ligand gated ion channel receptor and is influenced by diverse chemical ligands. Many drugs including benzodiazepines, alcohol, neurosteroids and inverse agonists modulated $GABA_A$ receptor mediated physiological functions.

Key words: $GABA_A$ receptors, Ethanol, Benzodiazepines, Inverse agonist, neurosteroids

Introduction

γ-Amino butyric acid (GABA) is quantitatively the major inhibitory neurotransmitter of the mammalian central nervous system (1). Almost 60-75 % of the neurons in the central nervous system are GABAergic in nature and they are virtually absent outside the brain and the spinal cord. The early ionotophoretic studies with GABA indicated that it generally produced inhibitory hyperpolarizing response on neurons, which was competitively

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blocked by the alkaloid bicuculline. The hyperpolarizing response is due to an increase in the chloride conductance of the neuronal membrane allowing chloride ions to flow down their electrochemical gradient into the cell. GABA controlled the state of excitability of the brain areas and the ongoing level of neuronal activity is regulated by the balance between excitatory inputs (mostly glutamatergic) and inhibitory GABAergic activity. Increased levels of GABAergic activity produce CNS depression, sedation, amnesia and ataxia. Any attenuation of the GABAergic system, on the other hand results in arousal response, anxiety, restlessness, insomnia and excitation (1). A typical GABAergic synapse is shown in Fig.1

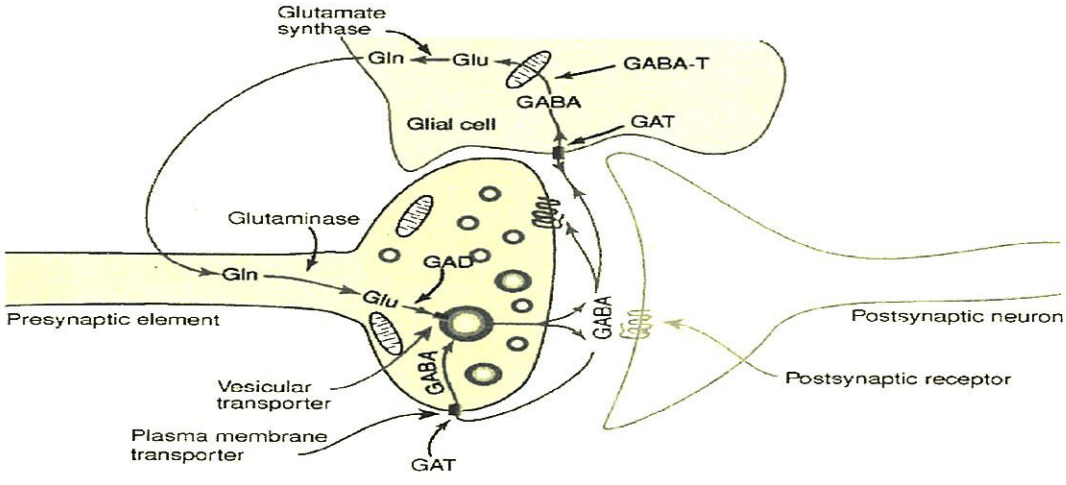

Fig. 1: $GABA_A$ ergic synapse

1. GABA receptors

GABA is known to act on three distinct receptors i.e. $GABA_A$, $GABA_B$ and $GABA_C$, respectively.

The $GABA_A$ receptor is a member of a superfamily of ligand-gated ion channels of the type of nicotinic acetylcholine, glycine, and 5 HT_3 receptors. $GABA_A$ receptor is a hetero-oligomeric (pentameric protein) complex in its native state, composed of subunits with molecular weight of ~ 40-60 kDa (2). At least 18 different receptor subunits genes have been separated into the sequence homology subfamilies alpha ($á_1$ - $á_6$), beta ($â_1$ to $â_3$), gamma ($ã_1$ - $ã_3$), delta, pi, epsilon, and theta, building a ligand gated chloride channel (3). Furthermore, two additional subunits ($â_4$, $ã_4$) of GABA receptors in chick brain and five isoforms of the rho-subunits in

the retina of the white perch have been identified. Most functional subtypes of $GABA_A$ receptor contain alpha, beta and gamma subunits. The preferred pentameric combination is either of two alpha, one beta and two gamma-subunits ($\alpha_2\beta\gamma_2$) or two alpha, two beta and one gamma ($\alpha_2\beta_2\gamma$) subunit. But one can expect a large number of combinatorial possibilities of $GABA_A$ receptors (Fig. 2).

α1	β1	γ1	δ	ε
α2	β2	γ2		
α3	β3	γ3		
α4	β4			
α5				
α6				

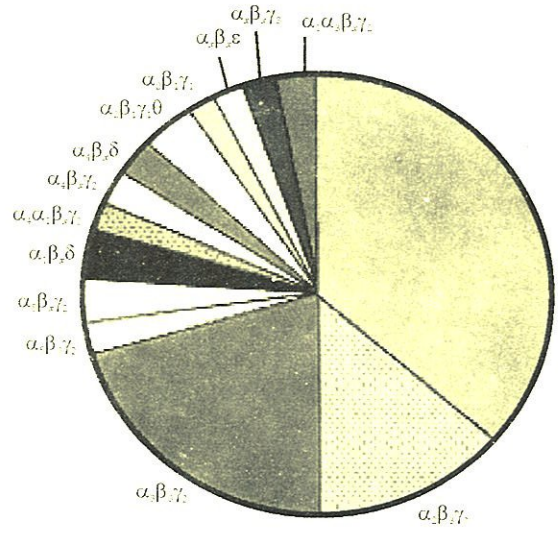

Figure 2 : Different protein subunits of $GABA_A$ receptor and various possible pentameric combinations of α, β and γ subunits of the receptor.

In the 1980's Bowery and his colleagues (4), observed that application of GABA reduced the evoked release of noradrenaline in the rat heart and this effect was not blocked by bicuculline. This action of GABA was mimicked by baclofen, 4-amino-3- (4-chlorophenyl) butanoic acid, a compound that had no effect on chloride conductance in central nervous system. This receptor was subsequently named as $GABA_B$ to differentiate it from its more familiar cousin, $GABA_A$ receptor. $GABA_B$ receptor is a metabotropic receptor, which are widely distributed within the central nervous system and also in peripheral autonomic terminals. The activation of $GABA_B$ receptors causes an inhibition of both basal and forskolin stimulated adenyl cyclase activity together with a decrease in Ca^{2+} and an increase in K^+ conductance in neuronal membranes. These receptors are activated by baclofen, and inhibited by phaclofen and saclofen. The $GABA_B$ receptor agonists are useful in the treatment of spasticity

pain and to reduce the craving for drugs of addiction. There is limited information on the therapeutic potential of $GABA_B$ receptor antagonists. The $GABA_B$ receptors are also functionally classified after the heterodimerization of $GABA_{(B1)}$ and $GABA_{(B2)}$ (previously known as GBR1 and GBR2). Both subunits are G-protein coupled receptors, a family of 7-transmebrane receptor family that shows a 30 % sequence homology to the metabotropic glutamate receptors (5, 6).

The third type of receptors namely $GABA_C$ are a distinct class of ligand gated ion channels that are activated by GABA. $GABA_C$ receptors are not blocked by bicuculline and do not recognize the benzodiazepines, barbiturates or the neuroactive steroids but, like $GABA_A$ receptors they are blocked by picrotoxin. The Table-1 summarizes the characteristics of different GABA receptor agonists and antagonists.

Table 1

Classification of GABA receptors: Molecular mechanism, Agonists and antagonists

	$GABA_A$	$GABA_B$	$GABA_C$
Effector Pathway	Ionotropic (ion channel) Cl^- ionophore	G- protein coupled (metabotropic) ↓ in Ca^{2+} and ↑ in K^+ conductance	Ion-channel receptor
Agonist	GABA, muscimol, many allosteric +ve modulators like benzodiazepines, barbiturates, neurosteroids, alcohol, general anesthetics, hypnotics, zolpidem	(-) Baclofen	Cis-4-aminocrotonic acid
Antagonists	Bicuculline, picrotoxin, pentylenetetrazol	Saclofen, phaclofen, CGP-35348, CGP55845A, CGP 54626	1,2,5,6-tetra hydropyridine-4-yl phosphinic acid (TPMPA)

2. *Functional consequences of $GABA_A$ receptors*

The $GABA_A$ receptor complex is one of the most versatile drug-responsive mechanisms in the body. Each subunit possesses a large extracellular N-terminus with a cysteine bridge (beta-loop transmembrane domains) (M1-M4), a large variable intracellular loop between M3 and M4 and a short extracellular C-terminus. The

extracellular N-terminus region contains three sites for N-linked glycosylation and the two-cysteine residues, which form the beta loop and may function in binding GABA and other ligands. The ends of the membranes spanning domains (M1-M4) are characterized by an abundant occurrence of positive charged amino acids, which are possibly involved in the regulation of chloride channels. The M2 domain, which is composed of several hydrophilic residues, is thought to line the channel pore. The extremely variable (length and composition) intracellular loop between M3 and M4 of some subunits contains consensus sequences for phosphorylation by protein kinase A and the tyrosine protein kinase (Fig. 3)

Activation of $GABA_A$ receptor by GABA leads to increased influx of chloride ions, resulting in membrane hyperpolarisation and neuronal inhibition.

A great number of distinct drug binding sites of the chloride channel have been characterized, each with its own set of agonists and antagonists (Fig. 4)

3. *Benzodiazepine receptors coupled to $GABA_A$ receptor*

The benzodiazepine receptors coupled to $GABA_A$ receptor domain are the site for at least three different types of ligand i.e. (1) diazepam-like (agonists) (2), opposite to diazepam (inverse agonists), and (3) neutral, having no direct effect, but antagonize diazepam

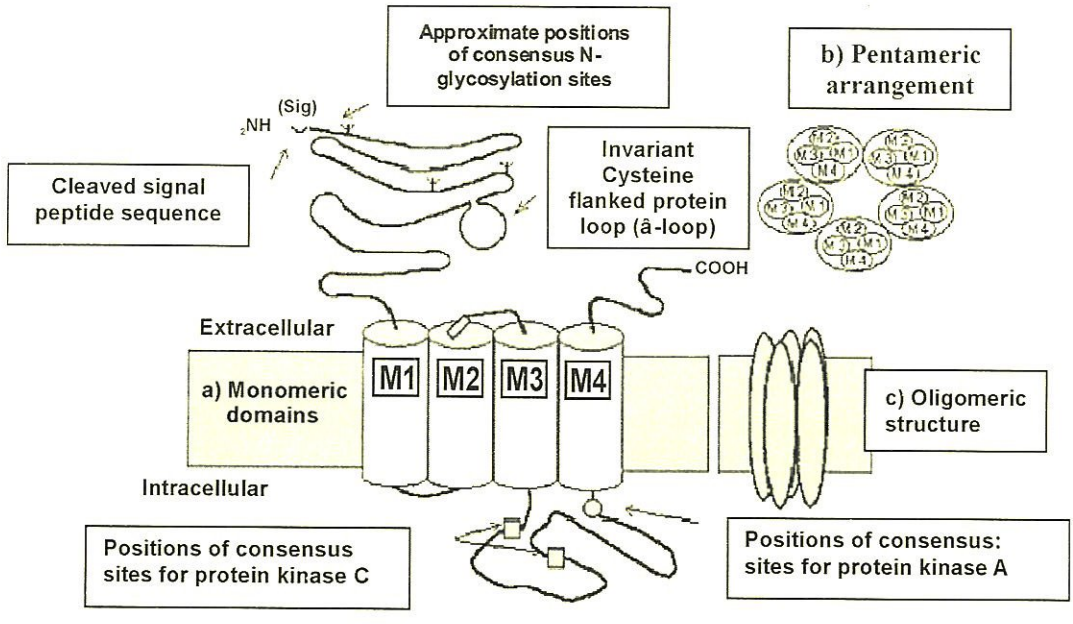

Figure 3: Model of $GABA_A$ receptor

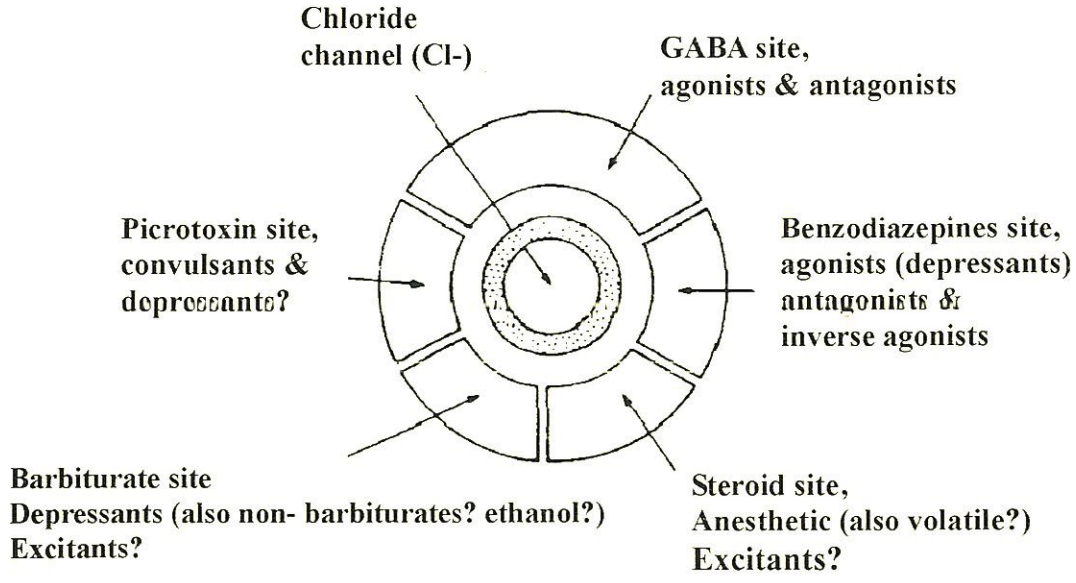

Figure 4: Various binding sites on the $GABA_A$ receptor Cl^- ionophore complex

effects (antagonists). The bidirectionality (agonists and inverse agonists) at the benzodiazepine receptor concept has been widely accepted and attempts have been made to explore the biological and clinical significance of endogenous ligands as well as drugs that modulated these two receptors state of benzodiazepines (Fig. 5a and Fig. 5b) (7,8).

4. Inverse agonist concept

The beta-carbolines, methyl-â-carboline-3-carboxylate (â-CCM), 6, 7 dimethoxy-4-ethyl-beta carboline-3-carboxylic acid methyl ester (DMCM), and N-methyl-beta-carboline, 3-carboxamide (FG-7142) act as inverse agonists or partial inverse agonists. These inverse agonists have the pharmacological effects that are opposite to full agonist namely diazepam, i.e. inverse agonists are anxiogenic, pro-conflict and pro-convulsants (9). Benzodiazepines and beta carboline (inverse agonist) recognition sites are located in the alpha subunits of the $GABA_A$ receptor and are organized into an allosteric modulatory centre (10, 11). This centre appears to exist in two interconvertible transitional states. One prefers anxiogenic beta carbolines and other prefers anxiolytic benzodiazepines. Injection of beta carbolines in humans causes a panic-anxiety disorder. GABA downregulates the binding of beta carbolines. When the centre is occupied by beta carbolines, the probability of action of GABA at $GABA_A$ receptor is reduced. Flumazenil, an

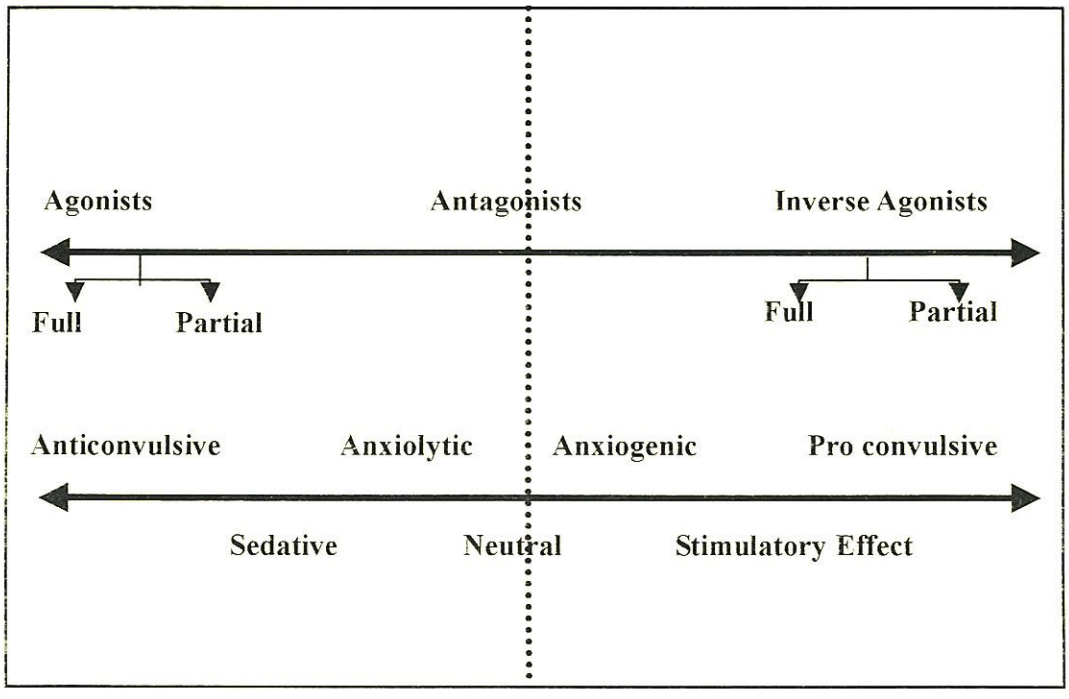

Figure 5a: Benzodiazepine receptor ligands and their functional consequences

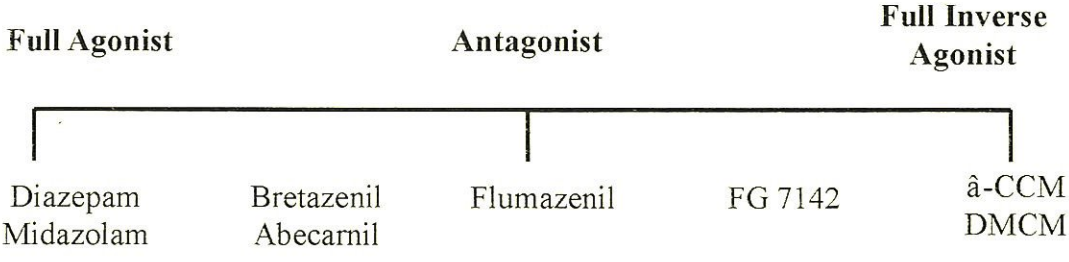

Figure 5b: Spectrum of activity of compounds acting at the benzodiazepine receptor

imidazobenzodiazepine, a benzodiazepine antagonist, antagonizes the action of beta carbolines as well as benzodiazepines. Fig. 6 illustrates the modulation of benzodiazepine receptor by agonists, inverse agonists and antagonist.

5. Ethanol and $GABA_A$ receptors

Ethanol has been reported to potentiate certain behavioral effects of GABA. Ethanol is also known to exert a number of behavioral effects such as sedation, ataxia, antianxiety, amnesia,

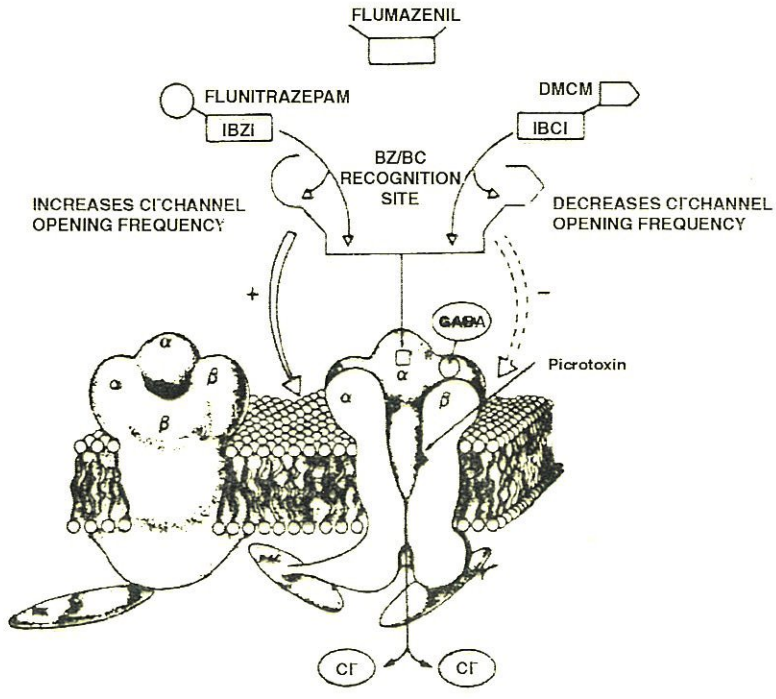

Figure 6: Benzodiazepine receptor and concept of agonist (benzodiazepines), inverse agonist (beta carbolines) and antagonist [BZ: Benzodiazepines; BC: beta carbolines]

muscle relaxation and anticonvulsant properties (Table 2) similar to diazepam. It has been speculated that the GABA-benzodiazepine receptor-coupled Cl^- channel could be the site of action of ethanol in the brain (7).

Ethanol is reported to potentiate GABA mediated Cl^- conductance. The formation of beta-carbolines is increased following ethanol and the urinary excretory pattern of these substances is correlated well with ethanol withdrawal syndrome, particularly anxiety and withdrawal convulsions. The inverse agonists favor the reversal of acute central nervous system depressant effect of ethanol and other depressants. With this background, several inverse agonists, both beta-carbolines and other benzodiazepines have been extensively investigated for their useful effect in reversing the acute intoxicating effects of alcohol. These compounds were thought to produce sobering effect in individuals intoxicated with ethanol (8).

Of particular interest is the imidazobenzodiazepine, R015-4513, a structural analogue of benzodiazepine receptor antagonist, flumazenil (R015-1788) which has been extensively investigated for anti-alcohol effects.

Table 2

Anticonvulsant effect of ethanol when studied against different chemoconvulsants in mice

Treatment (mg/kg, i.p.)	Onset (min ± SD)	Total seizure duration (min ± SD)	Mortality (%)
Bicuculline (4)	1.8 ± 0.8	6.4 ± 1.4	100
Picrotoxin (10)	5.0 ± 1.8	15.8 ± 3	100
Strychnine (4)	2.5 ± 0.5	5.2 ± 0.8	100
Ethanol (2 g/kg) + Bicuculline	2.3 ± 0.6	–	0
Ethanol (2 g/kg) + Picrotoxin	16 ± 1.0**	17.0 ± 3	70
Ethanol (2 g/kg) + Strychnine	2.3 ± 0.5	48 ± 27.0*	75

**P<0.01 and **P<0.001 as compared to corresponding per se effect of convulsant respectively*

6. Reversal of ethanol effect by R015-4513, an inverse agonist

R015-4513 is the azido analogue of the classic benzodiazepine receptor antagonist R015-1788 with ability to bind to benzodiazepine receptors. R015-4513 antagonized both *in vitro* ability of ethanol to stimulate $^{36}Cl^-$ uptake in rat brain synaptoneurosomes and *in vivo*, the anticonflict activity of low dose of ethanol as well as sedative effect of higher dose of ethanol in rats and mice. However, when administered alone, R015-4513 lowered seizure threshold to bicuculline, pentylenetetrazol, and R0 5-3663 and reduced explorative behavior in a hole-board apparatus (7).

7. Anti-alcohol pill: A myth or reality

Drugs possessing alcohol antagonistic properties have far reaching clinical application in the management of acute alcohol effect in man. The recent research findings with R015-4513 have raised wide speculations regarding the possible clinical use of this as well as other related drugs for sobering effects in alcohol intoxication. Rats passed out from alcohol intoxication acted sober within two minutes of R015-4513 administration. However, the possible clinical use of R015-4513 is limited due to its proconvulsant property (Table 3) (7), since it may precipitate withdrawal symptoms in alcoholics and also it has short duration of action (8).

Table 3

Proconvulsant effects of R015-4513 as studied against bicuculline

Treatment (mg/kg, i.p.)	Onset (sec ± SEM)	Mortality time (min ± SEM)	Mortality (/n)	Severity of convulsions
Bicuculline (1)	–	–	–	No convulsions
Bicuculline (4)	59 ± 5.77	14 ± 1.62	4/7	4/7 ++
Bicuculline (8)	36 ± 2.32	8 ± 0.63	10/10	+++
R015-4513 (1) + Bicuculline (4)	28 ± 2.25	15 ± 1.75	4/4	++
R015-4513 (1) + Bicuculline (8)	13 ± 3.0**	6 ± 0.84	6/6	+++
R015-4513 (4) + Bicuculline (8)	16 ± 1.35	2.8 ± 0.20*	5/5	+++

8. $GABA_A$ receptors and neurosteroids

Neurosteroids are the steroids that are synthesized within the brain either from cholesterol or from peripheral steroid hormones (12). Steroid hormones have long been recognized as possessing sedative and anaesthetic properties in animals and humans (13). Deoxycorticosterone, the first adrenal steroid isolated by Von Steiger in 1937 (14), has profound effect on CNS excitability besides having weak mineralocorticoid action. This neurosteroid can act as an endogenous anticonvulsant substance. Stress-induced hypothalamic corticotropin releasing hormone (CRH) stimulates adrenocor-ticotrophic hormone (ACTH), which in turn stimulates synthesis and release of cortisol and deoxycor-ticosterone from the adrenal cortex. During the course of stress response, the increased level of tetrahydrodeoxycorticosterone (THDOC) synthesized in liver and brain from circulating deoxycorticosterone, activates GABAergic inhibitory transmission in neuronal networks in hippocampus, amygdala, and cerebral cortex and controls seizure susceptibility (Fig. 7).

Studies indicate that antiseizure activity of deoxycorticosterone is due to its conversion to allotetrahydrodeoxycorticosterone (THDOC), a neurosteroid (Fig 7). Allopregnanolone and THDOC are generally viewed to share common physiological and pharmacological effects (anxiolytic, antiseizure, antistress, antidepressant, amnesic, and neuroprotection). Several studies have shown that neurosteroids are shown to produce pharmacological action by acting on $GABA_A$ receptors (15). The proposed molecular site for neurosteroid on $GABA_A$ receptor is shown in Fig. 8.

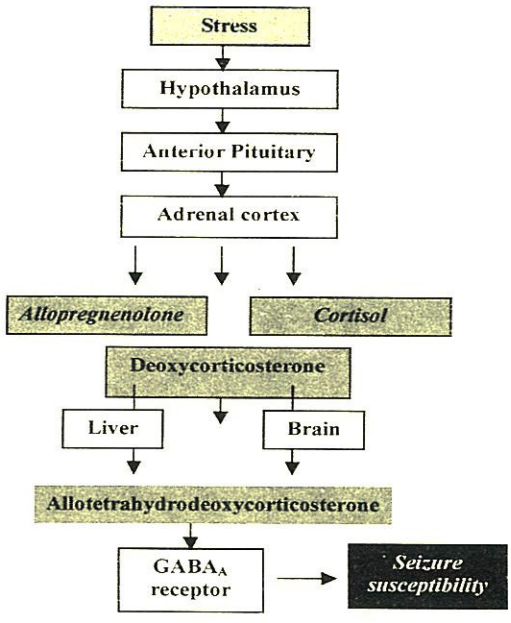

Fig. 7: Stress and activation of neurosteroids

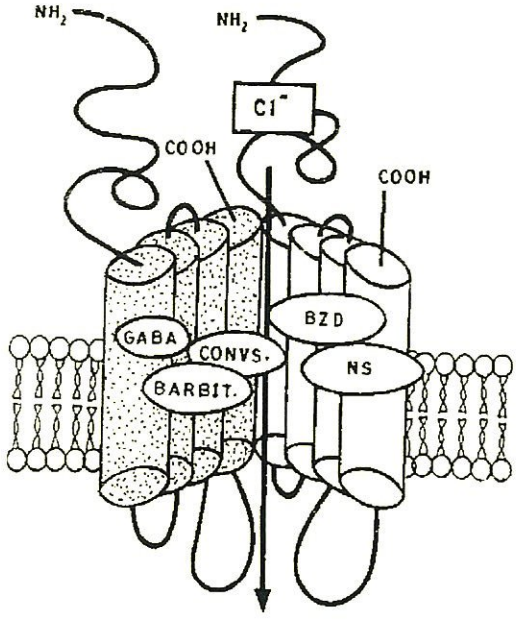

Fig 8: The possible binding site of neurosteroids (NS) on GABA$_A$ receptor

THDOC binds to GABA$_A$ receptor at site different from GABA, benzodiazepine or barbiturate. Several studies indicate that THDOC acts as positive allosteric modulator of GABA$_A$ receptor function and increases the inhibitory post-synaptic potential. THDOC potentiated the $^{36}Cl^-$ flux stimulated by GABA$_A$ receptor agonists. Neurosteroids enhancement of submaximal GABA$_A$ receptor function occurs through increase in both channel open frequency and open duration (16, 17).

Conclusion

GABA (A) receptor channels are ubiquitous in the mammalian central nervous system and there are rich chemical diversity of ligands that influence GABA$_A$ receptor function. Such diversity provides many avenues for the design and development of new chemical entities acting on GABA$_A$ receptors. Neurosteroids are one of the GABA$_A$ receptor modulators and found its usefulness in epilepsy and other CNS related disorders. RO 15-4513, an inverse agonist and similar drugs, selectively opposes some of the behavioral actions of ethanol could act as alcohol antagonist. The day is not far off when one would drive home safely from a party with too many drinks and an anti-alcohol pill!

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