DISCUSSION

The pharmacological receptors triggering the effects of benzodiazepines are localized in neuronal membranes as a part of an oligomeric complex with GABA<sub>A</sub> receptors and their associated chloride channel. The benzodiazepines are functionally involved in the coupling mechanism between GABA receptors and chloride channels. Benzodiazepines allosterically modulate the GABA receptor-effector system. However, they do not influence neuronal activity when the GABA-receptor-effector system is either in its resting state or maximally activated, since benzodiazepine receptor ligands do not affect chloride conductance. The GABA receptor antagonist can fully block the effects of benzodiazepines while the BZ receptor antagonist cannot block GABA
receptor agonistic effects. This indicates that benzodiazepines only modulate the efficiency of the stimulus provided by GABA.

Benzodiazepine receptors may contain recognition sites for three basic types of ligands; agonists, inverse agonists and antagonists (Fig. 36), each with different functional consequences. Agonists have anxiolytic, hypnotic, anticonvulsant and muscle relaxant properties and enhance the chloride conductance-increasing effects of GABA. Inverse agonists have anxiogenic and convulsant/proconvulsant properties and they reduce the effects of GABA. The competitive antagonists are inactive per se but prevent the receptor mediated effects of agonists and inverse agonists. The allosteric coupling explains the unique property of BZ receptors to mediate such a spectrum of effects with opposite efficacy (Richards et al., 1986).

It is also possible to rationalise the opposing effects of various BZ receptor ligands by assuming the presence of an endogenous ligand for the benzodiazepine receptor. One could assume that the endogenous ligand to be a partial agonist,
Fig. 36. Possible sites of action of BZ agonists, inverse agonists and competitive antagonists and modulation of GABA receptor action.

AGONISTS

INVERSE AGONISTS

COMPETITIVE ANTAGONISTS

DMCM

Ro 15-1788

CLONAZEPAM

GABA RECEPTOR

BZ RECEPTOR
imposing some tone on the receptor system. Displacement of this ligand with a compound of lower efficacy would result in that tone being decreased, while displacement with a ligand of greater efficacy would produce the opposite effect. The BZ receptor then could be seen as a true modulator of the GABA receptor, increasing or decreasing the result of a given GABA stimulus depending on its occupancy (Martin, 1987).

An alternative three state model has also been put forward (Poc et al., 1982; Nutt et al., 1982; Jensen et al., 1983). It assumes that the BZ receptor exists in three energy states. The first when occupied results in no effect being observed on the GABA activation of chloride channel opening - the antagonistic state. The second occupied by the agonist, results in an increased coupling between GABA and its effector mechanism; while the third state is preferred
when the receptor is occupied by the inverse agonist, resulting in decreased coupling between GABA and chloride ion channel.

GABA is a neurotransmitter at about 30% of the central synapses. GABA decreases the firing of CNS neurons due to hyperpolarizing response of an increase in membrane conductance to chloride ions. GABA is an inhibitory transmitter producing both pre- and post synaptic inhibition. In presynaptic inhibition the inhibitory synapses are located on the presynaptic nerve endings of excitatory neurons whereas the postsynaptic inhibition is mediated by synapses located on the postsynaptic membranes. GABA produces inhibitory responses by increasing the chloride permeability, leading to hyperpolarisation of the target cells (Ticku and Maksay, 1983). Benzodiazepines thus play an important role as modulators of GABAergic system.
Benzodiazepines, particularly, diazepam, are one of the most widely prescribed drugs. They are at one time considered to be the most safest CNS depressant drugs without any abuse liability. But recent studies have shown that they are capable of producing physical dependence with characteristic withdrawal syndrome in experimental animals and in man (Hallstrom and Lader, 1981; Petursson and Lader, 1981; Tyrer et al., 1983; Murphy et al., 1984). The severity of withdrawal reactions depended on the dose and duration of treatment (Boisse et al., 1986). Withdrawal reactions tend to be severe and occur earlier in patients taking benzodiazepines of short half-life, particularly triazolam and lorazepam (Morgan and Oswald, 1982) and considerable cross-tolerance existed between the benzodiazepines (Aranko et al., 1983). The withdrawal symptoms to benzodiazepines are reported to be increased anxiety, irritability, sleep disturbances, increased muscle tone and sexual function, multifocal myoclonus and convulsions (Nutt, 1986).
There is substantial evidence in clinical therapy that psychic dependence develops to the benzodiazepines as it does to other hypno-sedatives. However, it is difficult to demonstrate the phenomenon, as often spontaneous withdrawal of drug therapy with BZs produced anxiety state which is rather confined with the original symptoms for which anxiolytic medication was originally initiated. Experimentally also it is difficult to demonstrate spontaneous withdrawal reactions. With the availability of BZ antagonists it has been possible to precipitate some of the withdrawal symptoms. Within several minutes after the administration of Ro 15-1788, a BZ antagonist, signs of withdrawal stimulation such as hypermotility and increased muscle tone have been reported in rats, cats, baboons and monkeys (Lukas and Griffiths, 1982). Similar effects have been reported with yet another antagonist namely CGS 8216 in laboratory animals (McNicholas et al., 1983). However, the quantification of such withdrawal symptoms due to benzodiazepines has been difficult and no single biochemical
parameter has been described to be affected although recently changes in plasma corticosterone has been proven to be a sensitive and reliable indicator of acute and chronic dependence to narcotics and benzodiazepines (Eisenberg, 1987). In the present study, we have tried to quantify BZ withdrawal-induced hyperlocomotion and also correlate the same with changes in brain monoamine levels (second section).

On abrupt termination of chronic diazepam treatment, withdrawal symptoms were noted in rats which were mainly manifested as increased horizontal activity, vertical activity and diarrhoea. Increased anxiety has been reported with diazepam withdrawal in patients treated with 5 mg/kg diazepam for six weeks (Murphy et al., 1984). Since it is difficult to quantify the behavioural responses such as anxiety, in our study, we selected two variables which can be easily assessed using automated equipments. Withdrawal hyperactivity was measured as an increase in horizontal or vertical activity. Diarrhoea was seen as a prominent withdrawal symptom.
The peak withdrawal symptoms were seen on the third day of chronic diazepam termination. This could well be correlated with the long half life (20–70 hr) and high protein binding (96–98%) capacity of the drug (Williams et al., 1985).

Clonidine (50 μg/kg and 100 μg/kg) given twice a day at 12 hr intervals significantly reduced hyperactivity, as observed in the photoactometer and activity wheel tests following diazepam withdrawal. Treatment with guanfacine (106 μg/kg, twice daily) and B-HT 920 (106 μg/kg, twice daily) failed to show any such protective effect, although anti-diarrhoeal effect was seen. However, guanfacine and B-HT 920 were effective at the higher dose level (500 μg/kg, twice daily) in inhibiting withdrawal hyperactivity.

Clonidine is reported to be effective in alcohol (Wartenburg, 1983; Parale and Kulkarni, 1986) and opiate (Gold et al., 1978) withdrawal symptoms. Guanfacine and B-HT 920 are shown to be more specific in α2 receptor binding (Scholtysik, 1980; Kobinger and Pichler, 1980) but guanfacine and
B-HT 920 were found to be less effective than clonidine in the present study. This discrepancy could be due to a possible difference in their sites of action as well as in the pharmacodynamic properties of these agonists. Guanfacine is reported to have less sedative action as compared to clonidine (Scholtysik et al., 1975). The drugs also differ in their effects on dopamine turnover in rat brain (Scholtysik et al., 1975; Saameli et al., 1975). Topical application of clonidine to the exposed ventral surface of the medulla oblongata in cats induced a marked fall in the mean arterial blood pressure and heart rate (Bousquet and Guertzenstein, 1973; Scholtysik et al., 1975), while guanfacine was ineffective in this test model (Scholtysik et al., 1980), suggesting that the sites of action within the CNS may be different for the two drugs.

However, the alpha_2 adrenoceptor mediated effect of clonidine in diazepam withdrawal is supported by reversal of its effect by yohimbine pretreatment.
The sedative effect of clonidine, which is not seen with guanfacine and B-HT 920, does not seem to play a part in the protective effect. Moreover, the withdrawal symptoms were measured 2.5 hr after drug administration. There was no significant decrease in the parameters in control animals which received clonidine, guanfacine or B-HT 920, but an increase in the parameters was seen at certain intervals (Tables 15 & 16).

Withdrawal-induced diarrhoea was consistently abolished by all the three alpha₂ agonists studied. The stimulation of intestinal alpha₂ receptors is reported to reduce the motility and secretion of the gastrointestinal tract (Dijoseph et al., 1984).

Long term daily injections of diazepam are reported to induce a selective subsensitivity to microionotophoretically applied GABA in serotonergic dorsal raphae neuron in the rat, which may be reversed by Ro 15-1788 (Gallager et al., 1984; Gonsalves and Gallager, 1985). This subsensitivity persisted for 96 hr after the final dose of diazepam, eventhough the agonist and its active metabolites were no longer detectable in brain tissue. Martin et al. (1982)
reported that the course of the diazepam abstinence syndrome in rats roughly parallels the time course of reversal of the subsensitivity to GABA. Thus it is speculated that the decreased responsiveness to GABA in the absence of diazepam may contribute directly or indirectly to the behavioural and neurological signs of hyperexcitability seen after abrupt discontinuation of high doses of benzodiazepines (PeVnich et al., 1978; Winokur et al., 1980; Martin et al., 1982; Ryan and Boisse, 1983; McNicholas et al., 1983).

Modulation of the GABAergic system by alpha₂ adrenoceptors is not known. However, the involvement of a non GABAergic mechanism in benzodiazepine withdrawal is possible (Fariello and Ticku, 1983); if only secondary to altered GABA function. Since clonidine, guanfacine and B-HT 920 are shown to be effective in diazepam withdrawal hyperactivity, this suggests increased noradrenergic activity in diazepam withdrawal. However, this could be secondary to altered GABA function, as GABA has an inhibitory effect on the firing of noradrenergic neurons.
The biochemical data (as reported in the next section) showed a significant increase in brain monoamine levels, particularly NA and 5-HT, on diazepam withdrawal when compared with chronic diazepam-treated brain tissues of rats, further supporting the above observations.

AUTONOMIC HYPERACTIVITY ON DIAZEPAM WITHDRAWAL IN RATS

Incidence of diazepam withdrawal hyperactivity in rats has been reported in the earlier section. An adrenergic hyperactivity is documented in diazepam abstinence since alpha_2 adrenoceptor agonists such as clonidine, guanfacine and B-HT 920 were shown to be effective (earlier section). It was also noticed that the peak withdrawal symptoms occurred on third day of diazepam withdrawal.

In the present study, the heart rate as well as locomotion was monitored since the measurement of autonomic changes is shown to be more sensitive index than behavioural signs where an adrenergic hyperactivity existed (Buccafusco et al., 1984). A significant increase in heart rate was seen on
third day of diazepam withdrawal when compared with control group. Similarly a withdrawal hyperactivity was observed in the photoactometer studies on third day of diazepam termination. This supports our previous findings, indicating an adrenergic hyperactivity on diazepam withdrawal.

An increase in NA and 5-HT brain levels was seen in diazepam withdrawn rats when compared with control and with rats on chronic diazepam treatment indicating higher level of central autonomic function. The change in DA content could not be correlated with the activity (Table 17). Clonidine treatment did not influence NA level while DA and 5-HT levels were increased. The withdrawal induced hyperlocomotion was checked by clonidine treatment (Table 18). Clonidine as such is reported to lower the adrenergic activity through central mechanism (Kobinger, 1978; 1983). Hence the above observations support the fact of an increased noradrenergic function in diazepam abstinence in rats.
INvolvement of central type benzodiazepine and GABA<sub>A</sub> receptor in the protective effect of benzodiazepines in stress - induced gastric ulcers in rats

Stressors are known to induce gastric ulceration through multiple mechanisms (Mersereau and Hinchey, 1981; Morley et al., 1982). Diazepam (20 mg/kg) has been reported to be effective in 3 hr immobilization stress ulcers (Kunchandy et al., 1985). The present study also supports the previous finding: Besides diazepam, the other central type BZ receptor agonists, clonazepam and chlordiazepoxide also protected the animals from stress ulceration. Moreover, the protective effect of the central type BZ receptor agonists was reversed by pretreatment with specific central type BZ receptor antagonist, Ro 15-1788, suggesting the involvement of central type BZ receptor in their ulcer protective action. However, peripheral BZ receptor binding agent, Ro 5-4864 and BZ micromolar binding agent, phenytoin failed to show any protective effect.

Modulation of GABAergic system in BZ receptor action is well documented. Majewska and Chuang
(1985) reported that in glioma C₆ cells peripheral BZ receptors interact with GABAₐ type receptors. In human blood platelets, the peripheral BZ receptors are not coupled with any GABA binding site (Moi geon et al., 1984). The functional coupling of central type BZ receptor with GABAₐ receptors is also demonstrated (Hodges and Green, 1984; Quintero et al., 1985). Since bicuculline, a GABAₐ antagonist, not only reversed benzodiazepine effect but also aggravated stress responses in these animals, a modulatory role of GABAₐ in stress ulcers could be speculated. When a subeffective dose of diazepam (5 mg/kg) was combined with specific GABAₐ receptor agonist, muscimol (0.1 mg/kg) an enhanced protection was observed. A similar potentiation was found with a subeffective dose of clonazepam (1 mg/kg). The potentiation was found to be bicuculline reversible. However, similar potentiation was not observed with baclofen, a GABAₐ agonist. These studies indicate that central type BZ receptors are functionally coupled with GABAₐ binding site on the receptor complex and modulation of this system is responsible for the ulcer protective effect of benzodiazepines.
NALOXONE-SENSITIVE AND GABA<sub>A</sub> RECEPTOR MEDIATED ANALGESIC RESPONSE OF BENZODIAZEPINES IN MICE

In the present study, all the central type BZ receptor agonists such as clonazepam, chlor-diazepoxide and diazepam, increased the pain threshold to radiant heat (Fig. 22). This analgesic effect of clonazepam, chlor-diazepoxide and diazepam was antagonized by Ro 15-1788, a central type BZ receptor antagonist (Hunkler et al., 1981), indicating the involvement of central type BZ receptor activation in the analgesic effect. On the other hand, the peripheral BZ-receptor ligand Ro 5-4864 and the BZ micromolar binding agent phenytoin were devoid of any analgesic response.

Pretreatment with naloxone (1 mg/kg) reversed the analgesic effect of all the central type BZ receptor agonists. This indicates the involvement of the opioid system in their action. Diazepam is reported to release endogenous opioids from the central nervous system. The anxiolytic effect, anti-conflict effect and the hyperphagic response of diazepam are found to be naloxone sensitive.
reversible (Stapleton et al., 1979; Duka et al., 1980; Duka et al., 1981; 1982). Chlordiazepoxide-induced increase in drinking behaviour has also been abolished by small doses of either naloxone or naltrexone (Cooper, 1982).

Biochemical studies revealed that acute treatment with diazepam in rats significantly alters the enkephalin level in certain brain areas (Duka et al., 1980; Harsing et al., 1982), but the involvement of specific BZ receptors and the GABAergic system was not clear. However, these data imply that benzodiazepines and opioid mechanisms interact together to produce certain effects of benzodiazepines. Since in the present study, a naloxone-reversible analgesia is produced, it is reasonable to speculate the involvement of endogenous opioid release in BZ-induced analgesia.

Modulation of the GABAergic system in BZ receptor action is well documented. Majewska and Chuang (1985) proposed that in glioma C6 cells peripheral BZ receptors interact with GABA_A type receptors. In human blood platelets, the peripheral BZ receptors are not coupled with any GABA binding
site (Moigeon et al., 1984). However, many workers have demonstrated that the BZ receptors are linked with GABA<sub>A</sub> receptors (Hodges and Green, 1984; Quintero et al., 1985). Diazepam enhancement of low affinity GABA binding to rat brain membranes was also demonstrated (Skerritt et al., 1982; Paldi-Haris et al., 1985).

In the present study, pretreatment with bicuculline, a specific GABA<sub>A</sub> antagonist, reversed the analgesic effect of all the central type BZ receptor agonists. Moreover, an enhanced effect was seen when a subeffective dose of clonazepam was given with a sub analgesic dose of muscimol, a specific GABA<sub>A</sub> agonist. No such potentiation was seen when clonazepam was combined with baclofen, a specific GABA<sub>B</sub> agonist. These data imply a role of GABA<sub>A</sub> type receptor activation in opioid release. Similarly, an enhanced effect was observed when a subeffective dose of diazepam was given with pentobarbitone, indicating a role of the GABAergic system in the action of diazepam.

Nitrazepam and lorazepam (5 mg/kg and 10 mg/kg) failed to show any analgesic effect. Nitrazepam is reported to have only very feeble
action on central type BZ receptors and also in enhancing GABA effect (Skerritt and MacDonald, 1984).

The analgesic dose of diazepam (10 mg/kg) was tested for motor co-ordination using a rota-rod apparatus. The mice failed to show acute muscle relaxation as they remained on the rota-rod for 120 sec at 20 rpm, indicating that the reflex tail-flick action may not be affected by the drug at this dose level. Based on these observations, it is concluded that central BZ receptor agonists, clonazepam, chlordiazepoxide and diazepam, induce analgesia through a GABA_A sensitive opioid mechanism in mice.

MODULATORY EFFECT OF ALPHA-2 ADRENOCEPTOR AGONISTS ON Ro 5-4864-INDUCED CONVULSIONS IN RATS AND MICE

Ro 5-4864 (60 mg/kg) induced severe clonic-tonic convulsions and death in mice. Clonidine, ICI 106270, B-HT 920 and guanfacine, all alpha_2 agonists, exhibited anticonvulsant properties. Equidoses of B-HT and guanfacine were, however, less effective against Ro 5-4864-induced convulsions.
This discrepancy in action correlates with their potency as well as pharmacokinetic parameters. In in vivo studies, clonidine is reported to be the most potent alpha_2 adrenoceptor agonists even though guanfacine and B-HT 920 are more specific in alpha_2 receptor binding (Kobinger and Pichler, 1980; Scholtysik, 1980; Buccafusco et al., 1984; Kunchandy et al., 1985). In in vitro studies, ICI 106270 has been shown to have a specificity and potency equal to clonidine, but it was less potent when administered intravenously, indicating less penetration through the blood-brain barrier due to low lipophilicity. The pKa values for clonidine and ICI 106270 are 7.27 and 9.72 respectively, which suggests that the blood-brain barrier is less permeable to ICI 106270 as compared to clonidine (Ruffolo et al., 1984).

Pretreatment with the alpha_2 adrenoceptor antagonists yohimbine or idazoxan reversed the protective effect of clonidine and ICI 106270, indicating the involvement of alpha_2 adrenoceptors in their protective action.
Guanfacine and B-HT 920 have shown to be less potent than clonidine in many of their pharmacological actions (Buccafusco et al., 1984; Kunchandy et al., 1985). A similar profile of potency is found in the present study also. Moreover, their sites of action may also contribute to the variation in the observed biological response as described (earlier section).

Diazepam, clonazepam, CL 218,872 and pento-barbitone exhibited a different profile of protective action, as these agents protected the animals from death while alpha₂ adrenoceptor agonists were effective only in prolonging the latencies of jerk and convulsion. CL 218,872, which binds to the central BZ receptors, delayed the latency of jerk or convulsions and it significantly protected against mortality. ICV administration of clonidine and ICI 106270 was effective in delaying the onset of jerk and convulsion and also in protecting the rats from death. These differences in protective effect of alpha₂ adrenoceptor agonists, benzodiazepines, CL 218,872 and barbiturates indicate that Ro 5-4864 may have multiple sites for its convulsant action. A similar speculation has already
been made by other workers (Weissman et al., 1983; 1984; Pellow and File, 1984). Ro 5-4864 is reported to act at the picrotoxinin site of GABA-BZ receptor complex (Ticku and Ramanjansyulu, 1984) and is also known to potentiate the anxiogenic and convulsant action of picrotoxin (File and Pellow 1983; et al., 1984). Pentobarbitone also showed protective effect which again shows that Ro 5-4864 may be interacting with GABA-BZ receptor complex. However, we failed to replicate the protective effects of phenytoin (10-30 mg/kg) and Ro 15-1788 (3-20 mg/kg) as reported by File and Mabbutt, (1983). Similar observations have been reported by Weissman et al. (1984) who failed to observe the protective effect with Ro 15-1788. Animal strain, dose and the time of experimentation may contribute to these varying effects. The anxiogenic and convulsant actions of BZ inverse agonist are shown to be antagonized by Ro 15-1788. Here the convulsant effect of Ro 5-4864 was not blocked by Ro 15-1788, indicating that Ro 5-4864 is not an inverse agonist exerting its effects through the BZ receptors. This supports the picrotoxinin site of action of Ro 5-4864.
The exact anticonvulsant mechanism of clonidine remains to be speculative. Clonidine is reported to have benzodiazepine-like effects in many studies (Kulkarni, 1981; Kulkarni and Nagrath, 1983; Kunchandy et al., 1985). Our earlier studies have suggested a modulatory effect of alpha_2 adrenoceptors on GABA function (Earlier section). However, the doses of clonidine and ICI 106270 which showed significant effects in the present study ranged from 0.5 to 1 mg/kg. These data imply that alpha_2 adrenoceptor-mediated protection in Ro 5-4864-induced seizures may be due to altered GABA function. Multiple sites for Ro 5-4864-induced seizures and death can be expected, since a different profile of protective action is seen among the alpha_2 adrenoceptor agonists, benzodiazepines, CL 218,872 and barbiturates.

HYPOXIC STRESS-INDUCED CONVULSION AND DEATH: PROTECTIVE EFFECT OF ALPHA-2 ADRENOCEPTOR- AND BENZODIAZEPINE RECEPTOR AGONISTS AND Ro 5-4864

Physiological stressful situations are known to activate adenohypophysial axis and influence sympathetic discharge (Saley, 1952). More recently
stress is also known to stimulate the release of endopioids and GABAergic system. The stress-induced changes alter the cerebral oxidative metabolism and cerebral circulation (Rossingmol and Ebigei-Ibru, 1980; Spagnoli and Tognoni, 1983). The physiological changes that are seen due to acute hypoxia, like convulsion and death are mainly due to cerebral hypoxia. Alpha₂ adrenoceptor agonists such as clonidine, guanfacine, B-HT 920 and ICI 106270 shared significant protective effect. Pretreatment with yohimbine reversed the protective effect of clonidine. Their effect appears to be centrally mediated but whether the delay in the onset of convulsions is due to their anticonvulsant property or due to inhibition of sympathetic discharge is to be debated. However, the animals could significantly withstand the hypoxic stress after alpha₂ adrenoceptor agonist treatment. The prevention of stress motivated defecation could be due to their antidiarrhoeal property (Di Joseph et al., 1984; Kunchandy et al., 1985).

Alpha₂ adrenoceptors decrease the sympathetic tone thereby inducing vasodilation leading to increased perfusion (Kobinger, 1978; Rockhold and
Thus the improvement in cerebral perfusion may be one of the mechanisms of the protective effect of alpha\textsubscript{2} adrenoceptor agonists. The data presented in the earlier section suggests that centrally located alpha\textsubscript{2} adrenoceptors have a facilitatory effect on GABA function. Hence, the GABA facilitatory effect may also contribute to the antistress effect of clonidine in a similar fashion as that of GABA-ergic agents. Thus the vasodilatory effect as well as the GABA modulatory effect of alpha\textsubscript{2} adrenoceptors may be contributing to their protective action.

The classical antistress agents such as diazepam and CL 218872 also shared significant protective effect. Ro 5-4864, dose dependently delayed the latency of convulsion and death. Ro 5-4864 is reported to act through the peripheral type (Syapin and Skolnick, 1979; Shoemaker et al., 1981; Richards et al., 1982) and micromolar benzodiazepine sites (DeLorenzo et al., 1981; Bowling and DeLorenzo, 1982). It is also reported to be anxiogenic and convulsant (File and Nabbitt, 1983) acting at the picrotoxinin domain of BZ-GABA complex (Ticku and Ramanjaneyulu, 1984). However, benzodiazepine-like
action of Ro 5-4864 are reported. Ro 5-4864 displayed a sedative profile in hole board test (Pellow and File, 1984). In monkeys, Ro 5-4864 was equally effective as chlordiazepoxide in the continuous avoidance procedure (Pellow and File, 1984). In the present hypoxic model, Ro 5-4864 was found to be as effective as diazepam in delaying the onset of convulsion and death.

Piracetam is known to protect the brain against hypoxia (Nikolova et al., 1984) mainly by improving cerebral circulation (Vlahob et al., 1980), brain nucleotide and RNA metabolism (Tuneva et al., 1979) as well as the functional state of brain cortex in cats (Dimov et al., 1984).

Vasodilators like PGI are also reported to have antihypoxic effect (Nikolova et al., 1984). Several calcium entry blockers are reported to be effective in models of brain hypoxia (Wauquier, 1982; Wauquier et al., 1985). Recent investigations suggest that the peripheral type and the micromolar type benzodiazepine receptors, where Ro 5-4864 is an agonist, are involved in the modulation of Ca$^{++}$
channel function (Taft and DeLorenzo, 1984; Bender and Hertz, 1985; Mestre et al., 1985; Rampe and Triggle, 1987). Ro 5-4364 is shown to block the fast $^{45}\text{Ca}^{++}$ uptake in K$^+$ depolarised synaptosomes (Rampe and Triggle, 1987). Moreover, other benzodiazepines are also shown to have Ca$^{++}$ channel blocking effects (Taft and DeLorenzo, 1984; Rampe and Triggle, 1986). Such effects on Ca$^{++}$ flux can stabilize the CNS neurons as well as cause dilation of blood vessels thereby improving cerebral circulation. Morgan et al. (1983) have shown that Ro 5-4864 can affect adenosine accumulation, which is potent local vasodilator. In certain animals like monkeys, Ro 5-4864 is suggested to be converted to an active metabolite with effects similar to benzodiazepines. Such a pharmacokinetic probability as well as the modulatory effect on Ca$^{++}$ channel function and adenosine accumulation may explain the protective effect of Ro 5-4864 in hypoxic stress.
ON THE CONVULSANT ACTION OF DMCM AND Ro 5-4864
IN MICE

The peripheral BZ receptor agonist, Ro 5-4864 produced severe clonic-tonic convulsions. The myoclonic jerks were followed by severe convulsion characterised by rotational movements, violent abdominal writhing, chewing response and profused salivation. On the other hand, the BZ inverse agonist, DMCM convulsions were of intermittent clonic type of jerks followed by tonic convulsions. Pretreatment with alpha_2 adrenoceptor agonists shared significant protective effect as the latency for jerk and convulsion are concerned. Similar treatment with the potent alpha_2 adrenoceptor agonist, clonidine, failed to exhibit any protective effect in the DMCM-induced convulsions. However the benzodiazepine receptor antagonist, Ro 15-1788, antagonized the convulsant action of DMCM but not that induced by Ro 5-4864.

Ro 5-4864, chlorodiazepam, is reported to be a selective ligand for the GABA independent peripheral and micromolar binding sites (Braestrup and
Squires, 1977; DeLorenzo et al., 1981) but the pharmacological relevance of these sites is still not clearly defined. However, convincing evidences suggest a regulatory role on Ca\(^{++}\) channel function by these BZ sites (Rampe and Trigg\(\acute{\text{l}}\)e, 1987). PK 11195, the peripheral BZ receptor antagonist, reversed the anxiogenic and the convulsant actions of Ro 5-4864 (File and Pellow, 1983; File, 1984). The involvement of micromolar BZ site in Ro 5-4864 has been ruled out since phenytoin which has high affinity for the micromolar BZ site (Bowling and DeLorenzo, 1982) failed to protect the animals from Ro 5-4864-induced seizures (previous section). But the electrophysiological evidences suggest the involvement of GABA-BZ chloride ionophore complex in Ro 5-4864 actions. Ticku and Ramanjaneyulu (1984) have shown that Ro 5-4864 can displace \(^{35}\)S-TBPT from the picrotoxinin domain of GABA-BZ complex. Similar conclusion of the involvement of picrotoxinin site was also reported (previous sections). Hence, in the light of the pharmacological, electrophysiological and radioligand studies, it may be concluded that the convulsant
action of Ro 5-4864 is mediated through the picrotoxinin site in the GABA-BZ complex.

Apart from the picrotoxinin site, another convulsant site has been demonstrated in the GABA-BZ complex. The BZ receptors are speculated to contain recognition sites for three types of high affinity exogenous ligands with different pharmacological profile. The agonists, anxiolytic and anticonvulsant, are the positive allosteric modulators of GABA receptors thus enhancing the GABA mediated chloride conductance. The inverse agonists, anxiogenic and convulsant, are the negative allosteric modulators of GABA receptors, thus reducing the GABA mediated chloride conductance. The competitive antagonists are practically inactive per se but abolish the BZ receptor-mediated pharmacological effects of agonists and inverse agonists (Richards et al., 1986). Thus the BZ receptor probably contains a convulsant as well as an anticonvulsant site which modulate GABA mediated chloride flux.
Ro 5-4664-induced seizures are not antagonized by the BZ receptor antagonist, Ro 15-1788, indicating that the convulsant site of BZ receptor is not involved while the convulsant action of DMCM is fully antagonized by Ro 15-1788.

Clonidine is reported to have anticonvulsant effect against electro- and chemically-induced seizures (Kulkarni, 1981; Kulkarni and Mehta, 1983; Kulkarni and Nagrath, 1983). In the present study also clonidine and other alpha_2 adrenoceptor agonists shared significant anticonvulsant effect against Ro 5-4664-induced seizures. Similar activation of alpha_2 adrenoceptors failed to show any protection against DMCM-induced seizures. But the basic mechanism of DMCM- and Ro 5-4664-induced seizures are known to be due to reduction in GABA mediated chloride conductance (Pole et al., 1981b; Barker et al., 1984; Skerritt and MacDonald, 1984). However, the pathways or sites involved in modulating the GABA-ergic chloride conductance by Ro 5-4664 and DMCM appears to be different since the anticonvulsant profile of alpha_2 agonists were different in Ro 5-4664- and DMCM-induced seizures. Alpha_2 adrenoceptors have a modulatory effect on the pathway or
site of Ro 5-4864 seizures while no such effect could be seen in DMCM seizures. However, the differences in the site of action of these agents as well as the pharmacokinetic possibilities cannot be ruled out. The present study shows that alpha$_2$ adrenoceptors can modulate picrotoxinin site mediated GABA ergic effects while BZ inverse agonist mediated GABA ergic effects remain unaffected.

INTERACTION OF DIAZEPAM AND ADENOSINE IN PENTYLENE-TETRAZOLE-INDUCED SEIZURES IN MICE

Adenosine is reported to have anticonvulsant effect (Dunwiddie and Worth, 1982). Mehta and Kulkarni (1984) have shown that diazepam potentiated the relaxant effect of adenosine on rat caecum. Pretreatment with benzodiazepine receptor antagonist failed to antagonise the potentiating effect of diazepam, indicating a mechanism other than BZ receptor mediation may be involved in this action. The present in vivo experiment also showed potentiation of diazepam protective effect by adenosine. The
dose response curve of diazepam against pentylene- 
tetrazole-induced seizures was shifted to left 
by the subeffective dose of adenosine (50 mg/kg). 
This enhanced anticonvulsant effect may be due to 
the reported uptake inhibition of adenosine by 
diazepam. The specific adenosine uptake and metabolic 
inhibitor, dipyridamole significantly potentiated 
the effect of adenosine (50 mg/kg). The involvement 
of BZ receptor in the adenosine uptake inhibition by 
diazepam cannot be confirmed from the present 
in vivo study, since pretreatment with BZ receptor 
antagonist Ro 15-1788, would block the effect of 
diazepam as well. However, this potentiation was 
observed only in the latency for convulsion or death. 
The percentage mortality was unaffected by adenosine 
treatment.

APPARENT pA₂ ESTIMATION OF BENZODIAZEPINE RECEPTOR ANTAGONISTS

Ro 15-1788 and CGS 8216 are reported to be 
antagonists of BZ receptors. CGS 8216 is reported 
to have proconvulsant action at dose levels of 5-10 
mg/kg (File, 1983; Jensen and Petersen, 1983). The
doses (0.5 to 2 mg/kg) of CGS 8216 and Ro 15-1788 employed in our study were devoid of any proconvulsant action.

$pA_2$ is defined as the negative log of the molar concentration of the antagonist required to reduce the response of a full dose of the agonist to half. $pA$ values indicate the affinity constant of antagonists for the receptor and can be employed to determine and to compare the potency of various antagonists. Identical $pA_2$ values suggest the involvement of similar receptor types. In vivo $pA_2$ values have so far been reported for naloxone-neseroline (Furst et al., 1982), naloxone-morphine (Hayashi and Takemori, 1971), naloxone-gossypin (Viswanathan et al., 1984) and yohimbine-clonidine (Parale and Kulkarni, 1985) pairs. In the present estimation, the apparent $pA_2$ values for the Ro 15-1788-diazepam pair and CGS 8216-diazepam pair are found to be 5.28 and 5.31, respectively, which are not significantly different. This indicates that the BZ antagonists, Ro 15-1788 and CGS 8216 have almost equal potency at the BZ receptor site in reversing the anticonvulsant action of diazepam in rats.