AIMS AND OBJECTIVES

The main aims and objectives of the present study were (1) to study the pharmacology of benzodiazepine receptors, (2) establish their link with the GABA receptor complex in expressing pharmacological actions and (3) to study the interactions of benzodiazepine antagonists and inverse agonists with these receptors.

The experimental approach made to achieve the above said objectives is broadly discussed under the following heads. For the purpose of continuity the methods, results and discussion are described under these broad heads, namely (i) Reversal by alpha2 agonists of diazepam withdrawal hyperactivity in rats (ii) Autonomic hyperactivity on diazepam withdrawal in rats (iii) Involvement of central type benzodiazepine and GABA_A receptor in the protective effect of benzodiazepines in stress-induced gastric

REVERSAL BY ALPHA-2 AGONISTS OF DIAZEPAM WITHDRAWAL HYPERACTIVITY IN RATS

The benzodiazepine class of drugs are extensively used to treat anxiety, convulsions, sleep disorders, muscle spasm and alcohol withdrawal. They are believed to be the safest of the minor tranquillisers, although they do have potential for physiological and psychological dependence (Petursson and Lader, 1981; Tyrer et al., 1983). They are not only used concurrently with major tranquillisers and anti-
Depressants to alleviate anxiety symptoms associated with schizophrenia and depression but are also used chronically in the continued presence of anxiety and insomnia. Recent studies indicated that benzodiazepine treatment shows evidence of dependence (Hallstrom and Lader, 1981; Petursson and Lader, 1981; Tyrer et al., 1983) and withdrawal reactions on abrupt termination of chronic diazepam treatment in both humans and animals (Martin et al., 1982; Murphy et al., 1984). It was also observed that treatment with Ro 15-1788, a benzodiazepine receptor antagonist, precipitated withdrawal symptoms in baboons (Lamb and Griffiths, 1985) and in rats (Cumin et al., 1982; Lukas and Griffiths, 1982), indicating a physiological role of benzodiazepine receptors in the development of dependence.

Recently, clonidine has been advocated in opiate (Gold et al., 1978) and alcohol (Wartenburg, 1983; Parale and Kulkarni, 1986) withdrawal syndromes in human as well as in animals. In the present study, the antiwithdrawal effect of clonidine and two more alpha_2 agonists which are reported to be more specific in alpha_2 receptor binding than clonidine (Jarrot et al., 1982; Mottram, 1983) (Fig. 8) were investigated on diazepam withdrawal symptoms in rats.
FIG. 8 ALPHA2 ADRENOCEPTOR AGONISTS
Like other hypnotics and narcotics, benzodiazepines have the pharmacological potential to produce dependence and thereby withdrawal symptoms in experimental animals and human beings (Hallstrom and Lader, 1981; Tyrer et al., 1983; Murphy et al., 1984). Biochemical studies have shown that chronic diazepam treatment caused a selective subsensitivity to GABA in serotonergic dorsal raphae nucleus in rats which can be correlated with the abstinence syndrome (Martin et al., 1982). However, Gonsalves and Gallager (1985) reported that GABAergic and 5-HT mechanisms in dorsal raphae are not directly responsible for CNS hyperexcitability in diazepam abstinence. An adrenergic hyperactivity in diazepam withdrawal could be speculated. The possibility of such a non-GABAergic mechanism has been suggested (Cowen and Nutt, 1982; Fariello and Ticku, 1983). Recent studies have shown that central catecholamine release
plays an important role in the initiation and maintenance of opiate withdrawal symptoms (Aghajanian, 1978; Swann et al., 1983). Measurement of autonomic changes associated with abstinence syndrome would be more sensitive index than behavioural signs (Buccafusco et al., 1984).

In the present study, changes in heart rate and locomotion as diazepam withdrawal symptoms have been measured and an attempt has been made to correlate these activities with brain monoamine changes in the rat.

IN VolVEMENT OF CENTRAL TYPE BENZODIAZEPINE AND GABA
RECEPTOR IN THE PROTECTIVE EFFECT OF BENZODIAZEPINES IN STRESS-INDUCED GASTRIC ULCERS IN RATS

Modulatory as well as action interplay between benzodiazepines and GABA, an inhibitory neurotransmitter in the brain, have been described for many years (Costa et al., 1975; Haefely et al., 1979; Braestrup and Neilsen, 1982; Kulkarni and Jog, 1982). More recently, some biochemical studies have shown that GABA can potentiate the binding of radio-labelled benzodiazepines to brain membranes and,
conversely, benzodiazepines can potentiate GABA binding to such membranes (Braestrup and Neilsen, 1982; Morgan and Stone, 1982). These observations substantiate the concept of GABA receptor complex where BZ binding sites are coupled with GABA receptors through chloride channel mechanism (Olsen, 1982). Occupancy of GABA receptor by the neurotransmitter enhances chloride flux causing neuronal inhibition. Benzodiazepines influence this GABA mediated membranal change in chloride flux perhaps by acting on their binding sites in the GABA complex. In addition to these central inhibitory actions, peripheral type BZ receptors have been recently identified, whose physiological role is not yet fully defined, although specificity of agonists for central (clonazepam) and peripheral (Ro 5-4864) type of BZ sites have been demonstrated.

In the present study, an attempt has been made to study the involvement of GABA-BZ receptor modulatory phenomenon in the protective action of benzodiazepines against immobilization stress-induced gastric ulcers in rats.
Benzodiazepines are believed to exert their pharmacological effects through specific high affinity binding sites (Braestrup and Squires, 1977; 1978; File and Pellow, 1983). The binding sites of BZs have been recently classified as "central type" and "peripheral type". Ro 5-4864, a 1,4 benzodiazepine, is reported to have high affinity for peripheral type binding sites while it exhibited no affinity for the central type binding sites (File and Pellow, 1983; Weissman et al., 1984). Likewise, clonazepam binds preferentially to the central type receptors (File and Pellow, 1983). The central type receptors are located in various regions of the brain while peripheral type receptors are found in peripheral organs as well as in CNS (Braestrup and Squires, 1978; Gallager et al., 1981; Marangos et al., 1982; Richards et al., 1982). In addition, modulatory action of BZ receptors and GABA receptor complex is very well documented, as BZs selectively enhance postsynaptic GABAergic neurotransmission (Costa, 1983; Quintero et al., 1985).
Diazepam is also reported to act indirectly through endogenous substances like enkephalins (Duka et al., 1980; Harsing et al., 1982). In the present investigation, therefore, we have attempted to characterize the specific BZ receptor that might be involved in opioid release employing specific agonists and antagonists of peripheral, micromolar and central type BZ receptors in the analgesic action of BZs. Modulation by specific GABA receptor subtypes in the opioid release phenomenon was also investigated.

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

Clonidine, the centrally acting antihypertensive, is known to possess many extracardiovascular effects (Shearman and Lal, 1982; Kulkarni et al., 1984). It is reported to have sedative (Drew et al., 1979), anti-inflammatory (Kulkarni et al., 1986), anti-ulcer (Kunchandy et al., 1985), analgesic (Fielding et al., 1977) and anticonvulsant (Kulkarni, 1981) properties. The alpha_2 adrenoceptor agonists such as clonidine, guanfacine and BHT 920, have a modulatory
effect on GABA function as they are effective in diazepam abstinence syndrome (present study).
Clonidine has been reported to possess anticonvulsant properties against both drug- and electroshock-induced seizures (Kulkarni, 1981; Kulkarni and Nagrath, 1983; Kulkarni and Mehta, 1983). The anti-ulcer effect of clonidine in stress ulcers could be partially reversed by BZ antagonists (Kunchandy et al., 1985). All these observations suggest that the central alpha_2 adrenoceptors may have a modulatory role on GABA function in the CNS.

Ro 5-4864, a ligand for peripheral and CNS micromolar BZ binding sites, exhibits anxiogenic (File and Lister, 1983), sedative (File and Pellow, 1983) and convulsant (File and Mabbutt, 1983; Weissman et al., 1983) properties. Pharmacological and electrophysiological studies suggest that Ro 5-4864 interacts with the picrotoxin domain of GABA-benzodiazepine receptor complex (Pellow and File, 1984; Ticku and Ramanjaneyulu, 1984). In the present study, we have investigated the effect of various alpha_2 adrenoceptor agonists such as clonidine, guanfacine, B-HT 920 and ICI 106270 (Fig. 8) on
Ro 5-4864-induced seizures to explore the possible sites of interaction of these substances in the GABA-BZ receptor complex in the convulsant action of Ro 5-4864.

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

It is well documented that central nervous system is intimately concerned in the genesis of stress and behavioural changes that are seen due to stress. Multiple biochemical and physiological mechanisms are known to contribute to stress syndromes (File and Pearce, 1981; Morley et al., 1982; Bhargava et al., 1985). Recently, the benzodiazepine receptor inverse agonists such as beta-carbolines are reported to produce stress and related behavioural symptoms (Cowen et al., 1981; File et al., 1982; Ninan et al., 1982; Dorow et al., 1983), thereby suggesting the involvement of BZ-GABA ionophore complex in stress induced changes (File and Pearce, 1981; Bhargava et al., 1985; Havoundjian et al., 1986).
Central alpha<sub>2</sub> adrenoceptors are shown to have a facilitatory role in GABA function (present study). Moreover, clonidine and related drugs are known to possess anticonvulsant (Kulkarni 1981; Kulkarni and Mehta, 1983) and antistress effects (present study) akin to diazepam. Therefore, in the present study, we investigated the role of alpha<sub>2</sub> adrenoceptor and peripheral benzodiazepine receptor activation in hypoxic stress in mice and rats and their effects were compared with the effects of diazepam and CL 218,872, a BZ agonist.

**ON THE CONVULSANT ACTION OF DMCM AND Ro 5-4864 IN MICE**

Benzodiazepine receptor is the site of action of three classes of pharmacologically distinct agents. They are agonists, inverse agonist and competitive antagonist (Richards et al., 1986). DMCM is a BZ inverse agonist with anxiogenic and convulsant properties (Braestrup et al., 1982; Petersen, 1983). It is demonstrated that the inverse agonist reduces GABA ergic effects (Barker et al., 1984) leading to anxiety and convulsion. Ro 5-4864 is also a BZ receptor ligand preferring the peripheral type BZ
receptors, with anxiogenic and convulsant properties (File and Lister, 1983; Weissman et al., 1983). The centrally located alpha\textsubscript{2} adrenoceptors are shown to have a modulatory role on diazepam and Ro 5-4864-induced GABA ergic responses (Kulkarni, 1981; present study).

In the present study, the differences in the mechanisms of the convulsive actions of the BZ inverse agonist, DMCM and the BZ peripheral receptor agonist, Ro 5-4864 are elucidated, and also the protective effect of alpha\textsubscript{2} adrenoceptor agonists were studied as it has been extensively reported about the diazepam-like and anticonvulsant actions of clonidine, a prototype alpha\textsubscript{2} adrenoceptor agonist.

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

The basic mechanism of benzodiazepine action is shown to be mediated by enhancement of GABA ergic transmission (Haefely et al., 1975; Costa et al., 1975; Olsen, 1982). Benzodiazepines are also reported to have indirect actions. The central type BZ receptor agonists are reported to release endogenous opioids
thereby producing analgesic effect (Duka et al., 1980; Harsing et al., 1982; present study). It has also been suggested that benzodiazepine may act by potentiating the effects of adenosine, a mediator in the nervous system (Phillis, 1979; Phillis et al., 1980; Mehta and Kulkarni, 1984).

Adenosine has a powerful depressant action on the spontaneous activity of neurons in many regions of the brain (Phillis et al., 1974; Phillis and Kostopoulos, 1975; Kostopoulos and Phillis, 1977). Adenosine and its analogs are shown to produce depression in experimental animals (Kulkarni and Mehta, 1985) and is also reported to have anti¬convulsant and sedative properties (Dunwiddie and Worth, 1982).

Diazepam is reported to be a potent inhibitor of uptake of adenosine by brain slices and cultured glial cells and it potentiated the depressant action of adenosine on rat cortical neurons (Phillis, 1979). Moreover, purinergic substances such as ionosine, hypoxanthine are known to interact with benzodiazepine receptors at high concentrations (Skolnick et al., 1978; Marangos et al., 1979). In the present
investigation, we have attempted to demonstrate the adenosine uptake inhibitory effect of diazepam in its protective action against pentylenetetrazole-induced seizures in mice.

**APPARENT pA<sub>2</sub> ESTIMATION OF BENZODIAZEPINE RECEPTOR ANTAGONISTS**

Evidences suggest that benzodiazepines act on specific high affinity receptors to potentiate the inhibitory neurotransmission mediated by gamma-aminobutyric acid (Olsen, 1982). More recently, these BZ receptor sites have been classified as "central type" and "peripheral type". Clonazepam and Ro 15-1788 are shown to be the agonist and antagonist, respectively at the central type BZ receptors (Braestrup and Squires, 1977; Hunger et al., 1981; Polc et al., 1981a; Bonnetti et al., 1982) while Ro 5-4864 is reported to be an agonist for the peripheral type BZ receptor (Braestrup and Squires, 1977). In addition to Ro 15-1788, CGS 8216 (2-phenylpyrazolo (4,3c) quinolin-3-(5H)-one), a pyrazoloquinolone, is also reported to antagonize
the pharmacological effects of BZs (Bernard et al., 1981; Czernik et al., 1981; Hunkler et al., 1981; Richards et al., 1982; Yokoyama et al., 1982). Both Ro 15-1788 and CGS 8216 are known to precipitate BZ abstinence syndrome in experimental animals (Lukas and Griffiths, 1982; McNicholas et al., 1983). In addition to its antagonistic property, CGS 8216 is reported to have weak inverse agonistic action (File, 1983; Jensen and Petersen, 1983; Brown and Martin, 1985). In the present study, an attempt has been made to compare the in vivo antagonistic potency of Ro 15-1788 and CGS 8216 against diazepam-induced protection in pentylenetetrazole seizures in rats. The potency of these compounds is expressed using pA values.