SUMMARY AND CONCLUSION

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

Rats were made diazepam dependent by chronic treatment with daily injections of the drug, 20 mg/kg, ip, for three weeks. On abrupt termination of the drug, the animals showed withdrawal hyperactivity which was indicated by increased horizontal locomotion and vertical activity, and diarrhoea. The peak effect was seen three days after the withdrawal of chronic diazepam. Effects of various alpha₂ agonists such as clonidine, guanfacine and B-HT 920 were studied on the diazepam withdrawal phenomena. Clonidine (100 μg/kg, ip) given twice a day at an interval of 12 hr prevented both withdrawal-induced hyperactivity and diarrhoea. On the contrary, equimolar doses of
guanfacine and B-HT 920 failed to reverse withdrawal-induced hyperactivity but attenuated the effect of diarrhoea. However, higher doses (500 μg/kg, ip) of guanfacine and B-HT 920 given twice a day at 12 hr intervals were found to be effective. Pretreatment with yohimbine (1.5 mg/kg, ip) reversed the protective effect of clonidine, indicating the involvement of alpha₂ adrenoceptors in the anti-withdrawal effect of clonidine.

AUTONOMIC HYPERACTIVITY ON DIAZEPAM WITHDRAWAL IN RATS

Brain biogenic amines such as noradrenaline, dopamine, 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid were estimated on third day of chronic diazepam withdrawal as hyperactivity was noticed on the same day. The hyperfunction of adrenergic system in diazepam abstinence was indexed by monitoring the heart rate also. Significant increase in brain NA content and, locomotion and heart rate indicated a state of adrenergic hyperfunction in diazepam withdrawal. Clonidine (100 μg/kg, ip, twice daily) attenuated this observed hyperactivity.
Involvement of adrenergic system in diazepam abstinence syndrome is implicated.

INVOLVEMENT OF CENTRAL TYPE BENZODIAZEPINE AND GABA<sub>A</sub> RECEPTOR IN THE PROTECTIVE EFFECT OF BENZODIAZEPINES IN STRESS-INDUCED GASTRIC ULCERS IN RATS

Various benzodiazepines were studied for ulcer protective effect in immobilization stress-induced gastric ulcers in rats. Central type BZ receptor agonists such as clonazepam, chlorodiazepoxide and diazepam shared marked antiulcer effect. The ulcer protective action of these agents was reversed by pretreatment with Ro 15-1788, a central BZ receptor antagonist. However, peripheral BZ receptor binding agent Ro 5-4864 and BZ-micromolar binding agent phenytoin, failed to show any antiulcer effect. Pretreatment with bicuculline also reversed the ulcer protective action of central type BZ receptor agonists. A bicuculline sensitive potentiation was observed in the ulcer protective effect of central BZ receptor agonist, when a subeffective dose was combined with muscimol, a specific GABA<sub>A</sub> agonist. But no such potentiation was seen with baclofen, a GABA<sub>B</sub> agonist.
These data imply an interaction of central type BZ receptor with $GABA_A$ subtype receptor in the ulcer protective effect of benzodiazepines in rats.

**NALOXONE-SENSITIVE AND $GABA_A$ RECEPTOR MEDIATED ANALGESIC RESPONSE OF BENZODIAZEPINES IN MICE**

Various benzodiazepines were investigated for their analgesic effect by the tail-flick method in mice. Central type BZ-receptor agonists such as clonazepam, chlordiazepoxide and diazepam showed analgesia while the peripheral BZ-receptor binding agent, Ro 5-4864, and the BZ micromolar binding agent phenytoin, failed to show any effect. Other BZs, such as lorazepam and nitrazepam, were ineffective in producing analgesia. Pretreatment with naloxone antagonized the analgesic effect of central type BZ receptor agonists. Similarly, pretreatment with Ro 15-1788, a central BZ-receptor antagonist, also blocked the analgesic effect. When the involvement of the $GABA$ergic system in the analgesic response of BZ agonists was investigated, a potentiation of BZ action was seen as a combination of a subeffective
dose of clonazepam with a subanalgesic dose of muscimol, a specific $\text{GABA}_A$ agonist, showed an enhanced effect. Moreover, pretreatment with GABAergic substances like pentobarbital also showed a facilitatory effect on BZ-induced analgesia. On the other hand, a combination of clonazepam with baclofen, a specific $\text{GABA}_B$ agonist, failed to show any synergistic effect. The analgesic effect of central type BZ receptor agonists was found to be bicuculline reversible. It is concluded that $\text{GABA}_A$ receptor activation has a modulatory role in the naloxone sensitive analgesic effect of central type BZ receptor agonists.

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

The protective effect of various alpha$_2$ adrenoceptor agonists such as clonidine, guanfacine, B-HT 920 and ICI 106270 was investigated against Ro 5-4864-induced convulsions in mice and rats. Clonidine and ICI 106270 exhibited a profound anticonvulsant effect while equivalent doses of guanfacine and B-HT 920 were less effective. The anticonvulsant effect of clonidine and ICI 106270 was reversed by
pretreatment with yohimbine or idazoxan, indicating the involvement of alpha2 adrenoceptors in their protective effect. Diazepam, clonazepam, CL 218 872 and pentobarbitone exhibited a different profile of protective action, as these agents protected the animals from apparent mortality as compared to clonidine and ICI 106270 which prolonged the latencies of jerk and convulsion. Modulatory effects of alpha2 adrenoceptors in central GABA function and multiple sites for Ro 5-4864-induced seizures are suggested.

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

Various alpha2 adrenoceptor agonists such as clonidine, guanfacine, B-HT 920 and ICI 106270 were investigated for their effect in hypoxic stress in rats and mice. Pretreatment with alpha2 adrenoceptor agonists exhibited a yohimbine sensitive antistress effect as they prolonged the latency for convulsion and death due to hypoxia. Intracerebroventricular administration of clonidine and ICI 106270 also shared the protective effect, indicating
the involvement of central alpha\textsubscript{2} adrenoceptors. However, their protective effect though comparable to diazepam and CL 218872, was more pronounced. The peripheral benzodiazepine receptor agonist, Ro 5-4864, also exhibited profound antihypoxic stress effect. The central GABA facilitatory action and the vasodilatory effect of alpha\textsubscript{2} adrenoceptor agonists and the calcium channel modulatory effect and the effect on adenosine accumulation of Ro 5-4864 may be the mechanisms of protective action of these agents.

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

The difference in the mechanism of convulsant action of the benzodiazepine inverse agonist, DMCM and the peripheral type BZ receptor agonist Ro 5-4864 were elucidated in mice. DMCM and Ro 5-4864 produced clonic-tonic convulsions in mice at 25 mg/kg and 60 mg/kg dose, respectively. Activation of central alpha\textsubscript{2} adrenoceptors by clonidine significantly delayed the onset of Ro 5-4864-induced convulsions while no significant effect was seen in DMCM-induced seizures.
in mice. Pretreatment with Ro 15-1788 failed to antagonise the convulsant effect of Ro 5-4864. On the other hand, DMCM induced seizures were fully antagonised by the benzodiazepine receptor antagonist Ro 15-1788. These observations indicated that DMCM acted at the convulsive site of BZ receptor while Ro 5-4864 at some other site, probably, picrotoxinin site, which is modulated by alpha_2 adrenoceptors.

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

Various doses of adenosine (25-100 mg/kg) increased the latency of convulsion and death against pentylenetetrazole (PTZ)-induced seizures in mice. Adenosine failed to affect the mortality due to PTZ. Diazepam exhibited anticonvulsant effect as well as protected the animals against mortality. When a subeffective dose of adenosine (50 mg/kg) was combined with varying doses of diazepam, the dose response curve of diazepam was shifted to left, indicating potentiation. Pretreatment with dipyridamole, a
specific adenosine uptake and metabolic inhibitor, also potentiated the effect of adenosine in the above dose. These interactions studies suggested that like in the in vitro observation, diazepam may be inhibiting the uptake of adenosine and this could be the basis of its potentiating action.

**APPARENT pÅ ESTIMATION OF BENZODIAZEPINE RECEPTOR ANTAGONISTS**

The in vivo equivalent of pÅ values for benzodiazepine antagonists Ro 15-1788 and CGS 8216 were estimated using diazepam as agonist. ED$_{50}$ value of diazepam was established against pentylenetetrazole-induced seizures in rats. This value was raised by pretreatment with BZ antagonists Ro 15-1788 and CGS 8216, in a dose-dependent manner. The apparent pÅ values for Ro 15-1788-diazepam pair and CGS 8216-diazepam pair were found to be 5.28 and 5.31 respectively, which indicated almost equal antagonistic potency of these agents at the BZ receptor in reversing the protective effect of diazepam against pentylenetetrazole-induced seizures in rats.