Pakistan Journal Pharmaceutical Sciences
Vol. 10(1), January 1997, pp.1-8

NEUROCHEMICAL AND BEHAVIOURAL EFFECTS OF DIAZEPAM : EVIDENCES FROM ANIMAL MODELS

FARHAT BATOOL AND DARAKHSHAN J. HALEEM
Department of Biochemistry
Neurochemistry & Biochemical Neuropharmacology Research Unit
University of Karachi, Karachi-75270, Pakistan

ABSTRACT:

Neurochemical and behavioural research show that benzodiazepines (BZs) are the well-known anxiolytic drugs which are also used for the treatment of epilepsy, hypnosis and insomnia. Administration of BZs to experimental animals produces anxiolytic-like effects in various animal models and decreases exploratory activity. A role of serotonin (5-HT; 5-hydroxytryptamine) in both anxiolytic and anti-exploratory effects of BZs have been suggested. Drugs which mimick 5-HT function at the post synaptic sites have been shown to decrease anxiety in experimental animals. The present study analyses regionally specific effects of BZs on brain serotonin metabolism in relation to the reported behavioural and therapeutic profiles of the drugs.

INTRODUCTION

Early studies of the pharmacology of models of anxiety led to the suggestion that central serotonin (5-HT; 5-hydroxytryptamine) system is involved in responses to aversive events (Cook and Sepinwall, 1973; Stein et al. 1977) and that the anxiolytic effects of drugs such as benzodiazepines (BZs) resulted from a reduction in the activity of the serotonin system (Stein *et al.,* 1973). It has been suggested on the basis of previous behavioural and neurochemical studies that 5-HT could be considered as a central neurotransmitter involved in the modulation of anxiety and anti-anxiety effects of BZs (Apter and Greenberg, 1994). The advent of selective agonists and antagonists for 5-HT receptor subtypes has rekindled investigation of the role of 5-HT in anxiety mechanisms (Eison and Eison, 1994).

One of the proposed mechanisms of action for the anxiolytic effects of the BZs is via a decrease in central serotonergic neurotransmission (Wright *et al,* 1992). Experimental evidences suggest that overactivity of serotonergic pathways, particularly the ascending dorsal raphe system, may contribute to the production of pathological anxiety (Andrews and File, 1993). Further support for the hypothesis that anxiety originates from an overactivity of central 5-HT pathways stems from animal models, showing that inhibition of the 5-HT synthesis produces an anxiolytic

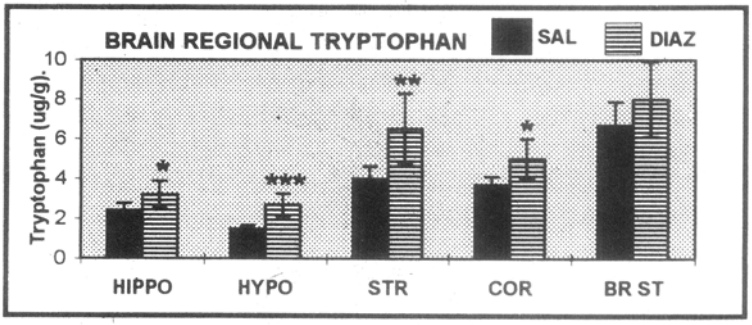
effect (Tenon, 1967) which can be blocked by 5-hydroxytryptophan (5-HTP; Wise *et a1.,* 1972).

New investigational pharmacological approaches to the treatment of anxiety focus mainly on the diazepam and serotonin functions (Barret and Vanover, 1993). With the identification of BZ receptors in the central nervous system (CNS; Mohler and Okada, 1977; Young and Kuhar, 1980) attempts have been made to prove that BZs bind to specific receptor in the brain and this results in decreased firing of serotonergic neurons (Trulson *et al.,* 1982). Another therapeutic profiles of BZs is the sedation. BZs are therefore, also used for the treatment of insomnia and epilepsy. The present article integrates knowledge from areas of neurochemistry and behavioural neuropharmacolpgy for an understanding of role of serotonin in the anti-anxiety and hypnotic effects of BZs.

NEUROCHEMICAL STUDIES:

Effects on Brain Tryptophan:

L-Tryptophan, an essential amino acid, is the precursor of serotonin. The initial source for the amino acid is dietry. Brain concentration of tryptophan is reported to decrease following the administration of diazepam (Valzelli *et al.,* 1980). In a dose related study, we observed an increase in brain regional tryptophan levels. However, regional variations were not observed in the effects of diazepam on brain tryptophan levels (see fig IA; Haleem and Batool, 1996). This suggest that administration of diazepam may inhibit the activity of tryptophan hydroxylase, the ram-limiting enzyme involved in the synthesis of 5-HT. The observed increases of free



(derived from Haleem and Batool, 1996, reference cited in the text)

Figure IA

(Elevated Levels of Brain Regional Tryptophan)

tryptophan levels at various doses of diazepam in the plasma are consistent with our previous work (Batool et al., 1994) and may be the result of displacement of tryptophan from its binding sites by diazepam (Muller and Wollert, 1975). Elevated levels of brain tryptophan may underlie enhanced availability of free tryptophan to the brain and the observed results are consistent with our previous work (see fig IB; Batool *et al.* 1994).

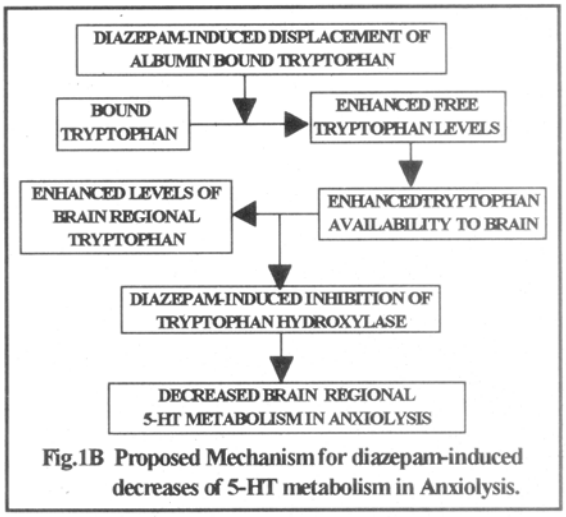
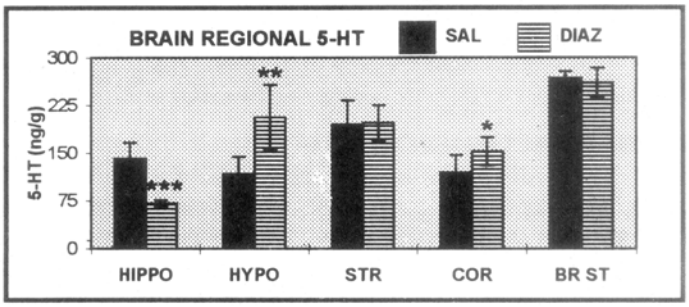


Fig.1B Proposed Mechanism for diazepam-induced decreases of 5-HT metabolism in Anxiolysis.

Effects on 5-HT and 5-HIAA :

Despite a fairly well documented role of 5-HT in the anti-anxiety effects of BZs, neurochemical studies on the effects of BZs on brain 5-HT metabolism/or turnover are not very consistent. On the basis of animal studies, it has been

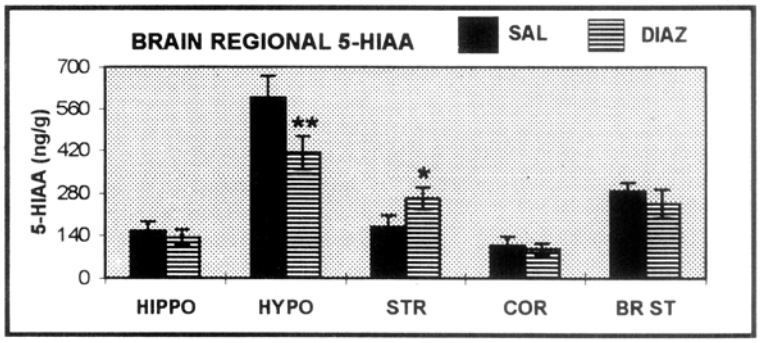


(derived from Haleem and Batool, 1996 and reference cited therein).

Figure 2

(Brain Regional Levels of 5-HT)

proposed that the sedative effects of BZs might be due to a reduction in noradrenaline transmission whereas the anxiolytic effects might be secondary to decreased 5-HT release (Wise et al., 1972). Animal studies have also shown that tolerance develops to some of these effects (Lister and File, 1983). Our results exhibited regional variations in the effects of diazepam on brain 5-HT and 5-HIAA levels. Thus administration of diazepam at doses of 1mg/kg decreased 5-HT levels in the hippocampus and increased it in the hypothalamus and cortex. The levels of 5-HIAA decreased in the hypothalamus and increased in the striatum (see fig 2 & 3). However, 5-HT (fig 2) and 5-HIAA (fig 3) levels are changed in a region specific manner (Haleem and Batool, 1996).



(derived from Haleem and Batool, 1996 and reference cited in the text)
Fig 3
(Brain Regional Levels Of 5-HIAA)

Evidences suggest that hippocampal changes may be involved in the anxiolytic effects of BZs. Serotonergic drugs have been shown to produce anxiolytic effects by stimulating presynaptic somatodendritic 5-HT receptors in the raphe nucleus resulting in an attenuation of serotonin neuronal firing particularly in the hippocampus (Andrew et al 1994). This is further supported by the fact that hippocampus is rich in BZ binding sites (Wameley et al., 1993).

NEUROPHARMACOLOGICAL STUDIES:

Anti-anxiety Effects:
Despite a recent trend of reduced prescribing, the BZs remain the most widely used psychotropic drugs (Petursson and Lader, 1984) and reflect their considerable effectiveness as anxiolytics (Rickets and Downing, 1966), hypnotics (Adam *et al.* 1976) and anticonvulsants (Feely *et al.* 1982).

Diazepam antagonists RO 15-1788, an imidazo-benzodiazepine derivative, identified by Hunkler (1981) and CGS 8216, a pyrazoloquinoline, identified by Yokoyama (1982) have been reported to antagonize all four of the principal actions of diazepam. In addition, another important factor is the difficulty in withdrawing them due to their tendency to produce abstinence reactions and dependence (Fontaine *et al.,* 1984) when used for longer periods. It is of interest that the selective BZ antagonists also prevented the increase in basal extracellular 5-HT produced by the exposure to the elevated plus maze test had no effect on behaviour. Pretreatment with the BZ antagonist, flumazenil (10-0mglkg) fully antagonized the behavioural effects of diazepam (Rex *et al.,* 1993).

EFFECTS OF SEROTONERGIG DRUGS IN ANXIETY STATES:

The hypothesis that the serotonin may he involved in the anti-anxiety effects of BZs originated from the *e*ar*ly* work by Geller and Blum (1970). The theory suggested that administration of para-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, produced anxiolytic effects in animal models of anxiety. This study was confirmed by Stein and Colleagues in 1973. The anxiolytic effect of PCPA was reversed by the administration of 5-hydroxytryptophan (5-HTP; an immediate precursor of 5-HT). Lesions of serotonin pathways in the brain provided further support for the involvement of 5-HT in anxiolysis. Intraventricular injection of 5,6 dihydroxytrptamine (5,6-D14T) and 5,7-DHT into the ventral tegamental area produced a marked effect and lesioning of dorsal raphe resulted in anxiolytic effect (Blackshear et *al.,* 1981).

It has been widely accepted that 5-HT neurons promote anxiety in human as well as in animal models. This could he termed the "classic hypothesis" and it has led to the determined search for drugs which reduce 5-HT function, especially agents which have selective actions at 5-HT receptor subtypes (Handley and McBlane, 1993). 5-HT antagonists like methysergide (5-HT-1, antagonist), cinanserine, cyproheptadine and metergoline (Cook and Sepinwall, 1975) have all been used in animal models of anxiety. A recent preliminary clinical trial of selective 5-HT-2 antagonist, seretiazine showed effectiveness against anxiety and depression (Janssen, 1983). Mianserin has anxiolytic properties in patients with mixed depression and anxiety (Brogden *et al.* 1978).

EFFECTS ON EXPLORATORY BEHAVIOUR:

Behavioural effects of diazepam generally thought to be a reflection of its anxiolytic properties. There is a wide range of animal models showing changes in activity in a familiar and novel environment (File *et al.* 1991). Depression of spontaneous locomotor activity following the administration of diazepam have been

shown in a number of animal sudies (Carmela and Hughes, 1996). Chronic administration of diazepam also decreases locomotion (Baldwin and Rudge, 1995). Our results show that both single and repeated administration of diazepam at doses of 1mg/kg decreases spontaneous locomotor activity in a novel (open field) as well as familiar (home cage) environment (Batool and Haleem, 1995).

CONCLUSION

In conclusion, the neurochemical and behavioural research reviewed in the present article suggests that systemically administered diazepam increases brain regional tryptophan levels. However, 5-HT and 5-HIAA levels are changed in a region specific manner. Diazepam-induced changes of brain regional 5-HT metabolism are explainable in terms of: (1). an increase in the availability of tryptophan to the brain possibly dike to an increase of plasma free tryptophan levels, (2). inhibition of serotonin release manifested by the facilitation of GABAergic neurotransmission and (3). interaction of BZs and/or 5-HT with other neurotransmitters. Evidences suggest that inhibitory effects of diazepam on particularly hippocampal 5-HT are anxiolytic (Handley and McBlane, 1993). Changes of 5-HT metabolism in other brain regions may well be involved in the neuroendocrine, anorectic and psychomotor effects of diazepam. The present contribution also focusses that single and repeated administration of diazepam resulted in depression of spontaneous locomotor activity in a novel as well as familiar environment due to sedation.

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