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DEVELOPMENT OF DIFFERENTIAL TOLERANCE TO THE SEDATIVE AND ANTI-STRESS EFFECTS OF BENZODIAZEPINES

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Abstract : Differential degree of tolerance has been reported to develop for anticonvulsant, sedative and skeletal muscle relaxant effects of benzodiazepines (BZDs). Acute treatment with BZDs reportedly reduces the formation of gastric stress ulcers and attenuates stress-induced immunosuppression. The present study investigates whether tolerance develops to these antistress effects of BZDs by using diazepam and chlordiazepoxide as representative drugs. A single dose of diazepam (5 mg/kg, ip) or chlordiazepoxide (20 mg/kg, ip) produced a significant reduction in locomotor activity, a measure of sedative effect and antagonized the effect of restraint stress (RS) on gastric mucosal lesions and anti-sheep red blood cell (SRBC) antibody titre. With chronic treatment (X 7 d), there was a marked tolerance to the sedative effect of both the studied BZD drugs, while much less tolerance developed to their ulcer protective action. However, no tolerance was observed to the attenuating effect of diazepam and chlordiazepoxide on RS-induced immunosuppression. Thus, the results of the present study indicate that different mechanisms may be involved in the development of tolerance to the sedative, antiulcer and immunomodulatory effects of BZDs.

Key words : diazepam, chlordiazepoxide, locomotor activity, gastric ulcers, tolerance, immune modulation

INTRODUCTION

Benzodiazepines (BZDs) have been widely used as sedative-hypnotic, antianxiety-antistress, anticonvulsant and centrally acting skeletal muscle relaxing agents (1). An undesired aspect of BZD therapy is the relatively rapid development of tolerance to their therapeutic effects especially to anticonvulsant, sedative and skeletal muscle relaxant actions (2). This tolerance in almost all cases is functional rather than dispositional (metabolic) in nature. The nervous system adapts so that it is no longer as responsive to subsequent exposure to a BZD agonist (3, 4).

Acute treatment with BZDs is reported to reduce the formation of gastric stress ulcers and attenuate stress-induced immunosuppression (5, 6). Whether tolerance develops to these antistress effects of BZDs is not clearly defined. The present study has been carried out to address this

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problem by investigating the effect of acute as well as chronic treatment with diazepam and chlordiazepoxide as representative of BZD group of drugs on locomotor activity, as a measure of sedative effect and stress-induced changes in gastric mucosal integrity and anti-SRBC antibody titre, as a measure of antistress activity.

METHODS

The study was carried out in male Wistar rats (150–200 g). Only male rats were used for the study, since in female animals estrus related differences in sex steroid levels have been reported to affect BZD-GABA receptor activity (7). The animals were procured from the local animal dealer and kept in light/dark cycles (12 h–12 h) and temperature-controlled conditions. Pellet diet (Brook Bond Lipton, India Ltd.) and water were available ad libitum, except when indicated otherwise. Animal care was as per the 'Guidelines for the Care and Use of Laboratory Animals' prepared by the Indian National Science Academy, New Delhi.

Locomotor activity: The test was carried out using an open field apparatus consisting of large circular enclosure having a white sunmica floor 85 cm in diameter and divided into 25 segments by intersection of 3 concentric circles and lines radiating from the centre. Animals were divided into different groups. First group was treated with the vehicle and II and III groups were injected with a single dose of diazepam (5 mg/kg, ip) and chlordiazepoxide (20 mg/kg, ip), respectively. Thirty minutes after drug/vehicle administration, the animals were placed individually in the centre of the apparatus and the number of floor segments entered by the animal over a period of 5 min was counted.

In another set of experiments the animals were chronically treated with vehicle, diazepam (5 mg/kg, ip) or chlordiazepoxide (20 mg/kg, ip) for 7 days. After a drug-free period of 48 h, on day 9 the animals were injected with the respective drug and 30 minutes later locomotor activity was measured.

Stress parameters: The animals were sensitized with sheep red blood cells (SRBC; 0.5×10^9 cells/ml/100 g, ip) on day 0 (sensitizing dose). On day 8, the rats were administered the same dose of antigen again (booster dose) and divided into different groups. Animals of group I were kept in home cages and not subjected to stress but were food and water deprived like stressed animals. The animals of II, III and IV groups were food but not water deprived for 24 h. On day 9, they were pretreated with vehicle, diazepam (5 mg/kg, ip) and chlordiazepoxide (20 mg/kg, ip), respectively and then subjected to restraint stress (RS) in plexiglas restrainers (INCO, Ambala) for 24 h at room temperature (22 ± 2°C). After the completion of stress procedure all the animals were mildly anaesthetized with ether and blood was collected from the retroorbital plexus. The serum was separated and analysed for haemagglutination titre (5).

After collecting the blood, the rats were sacrificed with an overdose of ether. The stomachs were dissected out, cut open along the greater curvature, washed with cold water and examined microscopically (X 10) under a dissecting microscope with a micrometer (Zeiss, GDR). The number of gastric mucosal lesions and their severity (cumulative length in mm to the nearest 0.1 mm) were determined.

In another set of experiments animals were sensitized as above but chronically treated with vehicle, diazepam (5 mg/kg, ip) or chlordiazepoxide (20 mg/kg, ip) from day 1 to 7. On day 8 they were injected with the booster dose of antigen. On day 9 they were administered with the respective drug and then subjected to RS for 24 h. After completion of stress procedure, the haemagglutination titre and gastric ulcerations were measured as above.

The data were analysed using Mann-Whitney 'U' test and a 'p' value of 'less than' 0.05 was used as the level of significance in all statistical tests.

RESULTS

A single dose of diazepam (5 mg/kg) and chlordiazepoxide (20 mg/kg) produced a significant reduction ($P<0.001$) in locomotor activity and antagonized the effect of RS on gastric mucosal lesions and anti-SRBC antibody titre (Table I and II). With chronic treatment there was a marked tolerance to the sedative effect of both the studied BZDs, i.e. reduction in the locomotor activity with an acute dose of respective drugs was much less marked in animals treated with chronic doses of BZDs as compared to the groups treated with the respective vehicle chronically.

Contrary to the sedative effect, less tolerance was observed to the ulcer protective effects of the used BZDs. Although in animals treated with chronic doses of both BZDs, there was a reduction in the number and severity of gastric lesions as compared to the acute control group, the extent of protection afforded after chronic treatment with BZDs, was significantly less when compared to acute treatment with single doses of both diazepam (75.2% vs 48.1%, cumulative length; 73.2% vs 41.1%, ulcer numbers) and chlordiazepoxide (68.7% vs 46.3%, cumulative length; 69.3% vs 43.4%, ulcer number). Unlike the development of tolerance of different magnitude to the effects of diazepam and chlordiazepoxide on locomotor activity and stress-induced gastric ulcerogenesis, no tolerance was observed to their attenuating effect on RS-induced immunosuppression, i.e. the antibody titre in animals chronically treated with diazepam or chlordiazepoxide was not significantly different from anti-SRBC antibody titre obtained in rats treated chronically with the respective vehicles (Table I and II).

TABLE I: Effects of diazepam (DZP) on locomotor activity and restraint stress (RS)-induced changes in gastric ulcerogenesis and immune responses.

Treatment (mg/kg, ip)	*Locomotor activity (counts/ 5 min)*	*Stress parameters*		
		Gastric ulcers		*Anti-SRBC antibody titre*
		Cumulative length (mm)	*Number*	
Acute				
Control	64.1±8.9	25.8±7.8	19.0±2.9	4.5±0.3
DZP (5)	20.7±4.5^{**a}	6.4±1.4^{**a}	5.1±1.2^{**a}	6.3±0.2^{**a}
Chronic				
Control [Vehicle × 7d + DZP (5) on day 9]	21.8±4.2^{**b}	6.8±1.6^{**b}	4.8±1.6^{**b}	6.5±0.3^{**b}
DZP (5) [DZP (5) × 7d + DZP (5) on day 9]	62.1±8.4^{**a}	13.4±1.9^{*ab}	11.2±1.2^{*ab}	7.3±0.7^{**b}

Values are mean ± SE (n = 8)
*P< 0.05, **P< 0.001
[a]Compared to respective control; [b]Compared to acute control.

TABLE II: Effects of chlordiazepoxide (CDP) on locomotor activity and restraint stress (RS)-induced changes in gastric ulcerogenesis and immune responses.

Treatment (mg/kg, ip)	*Locomotor activity (counts/ 5 min)*	*Stress parameters*		
		Gastric ulcers		*Anti-SRBC antibody titre*
		Cumulative length (mm)	*Number*	
Acute				
Control	49.5±3.8	26.8±6.8	21.2±2.8	4.0±0.4
CDP (20)	20.2±3.4**[a]	8.4±1.2**[a]	6.5±1.3**[a]	6.5±0.4**[a]
Chronic				
Control [Vehicle × 7d + CDP (20) on day 9]	20.8±4.1**[b]	8.2±1.3**[b]	6.4±1.6**[b]	6.3±0.5**[b]
CDP (20) [CDP (20) × 7d + CDP (20) on day 9]	56.8±7.6**[a]	14.4±1.6*[ab]	12.0±1.7*[ab]	7.0±0.6**[b]

Values are mean ± SE (n = 8)
*P< 0.05, **P< 0.001
[a]Compared to respective control; [b]Compared to acute control.

DISCUSSION

The results of present study show that marked tolerance developed to the effect of BZDs on the locomotor activity. Rapid development of tolerance to the sedative effect of BZD group of drugs has also been reported by other workers (2). Much less tolerance occurred to the ulcer protective effect of BZDs and no tolerance developed to the attenuating action of diazepam and chlordiazepoxide on RS-induced immunosuppression in the observed period. Down regulation of BZD receptors could be responsible for development of tolerance to some of the effects of BZDs (8). This down regulation of BZD receptors reportedly shows an anatomical regional variability (9). Since different brain areas are involved in mediating different functions, this anatomical regional variability in BZD receptor down regulation could be responsible for the observed differential pattern of development of tolerance.

BZDs are known to exert their effects by augmenting $GABA_A$-receptor mediated chloride ion channel conductance. Central BZD-$GABA_A$ receptor complex occurs as a supramolecular complex, comprising probably five subunits (α, β, γ, δ and ε) that form a Cl^- permeable channel (10–13). Multiple variants within the subunits have been shown to exist and sequence of six α, three β, two γ and one δ subunits have been reported (10–12). The exact pharmacology of the BZD response varies according to the α subunit expressed since the α subunit carries the BZD binding site (10–12). $GABA_A$ receptors with variable α subunit composition have been reported to exist in different brain regions. Hence, the observed differential development of tolerance to various effects of BZDs could possibly be due to heterogeneity of $GABA_A$ receptor subunits in the receptor complex involved in different actions in different areas (11, 12).

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology, 4th edn. New Delhi, Jaypee Brothers, 1999: 366–380.
2. Gallager DW, Wilson MA. Chronic benzodiazepine agonist exposure: comparison of eletrophysiological changes by brain region and benzodiazepine receptor ligand. In: Chloride Channels and their Modulation by Neurotransmitters and Drugs, Advances in Biochemical Psychopharmacology, Vol. 45, Biggio G, Costa E (eds). New York, Raven Press, 1988: 325–335.
3. Lister RG, File SE, Greenblatt DJ. Functional tolerance to lorazepam in the rat. *Psychopharmacology* 1983; 81: 292–294.
4. Rosenberg HC, Chiu TH. Nature of functional tolerance produced by chronic flurazepam treatment in the cat. *Eur J Pharmacol* 1982; 81: 357–365.
5. Ray A, Mediratta PK, Puri S, Sen P. Effects of stress on immune responsiveness, gastric ulcerogenesis and plasma corticosterone in rats: modulation by diazepam and naltrexone. *Indian J Exp Biol* 1991 ; 29: 233–236.
6. Mediratta PK, Sharma KK. Role of benzodiazepine-GABA receptor complex in stress-induced modulation of leucocyte migration inhibition factor *Indian J Pharmacol* 1997; 29: 228–232.
7. Finn DA, Gee KW. The estrus cycle, sensitivity to convulsants and the anticonvulsant effect of neuroactive steroid. *J Pharmacol Exp Ther* 1994; 271: 164–170.
8. Rosenberg HC, Chiu TH. Tolerance during chronic benzodiazepine treatment associated with decreased receptor binding. *Eur J Pharmacol* 1981; 70: 453–460.
9. Tietz El, Rosenberg MC, Chiu TH. Autoradiographic localization of benzodiazepine receptor down regulation. *J Pharmacol Exp Ther* 1986; 236: 284–292.
10. Guidotti A, Slobodyansky E, Berkovich A, Cox DH, Wambebe C. The heterogeneity of $GABA_A$ receptors and the multiplicity of endogenous ligands for their allosteric modulatory centres. In: Chloride Channels and their Modulation by Neurotransmitters and Drugs, Advances in Biochemical Psychopharmacology, Vol. 45, Biggio G, Costa E (eds). New York, Raven Press, 1988: 219–226.
11. Luddens H, Wisden W. Function and pharmacology of multiple $GABA_A$ receptor subunits. *Trends Pharmacol Sci* 1991; 12: 49–51.
12. Sieghart W. Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes. *Pharmacol Rev* 1995; 47: 181–234.
13. Seutin V, Johnson SW. Recent advances in the pharmacology of quaternary salts of bicuculline. *Trend Pharmcol Sci* 1999; 20: 268–270.