MATERIALS AND METHODS

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

Animals and techniques

Adult male Wistar rats weighing 200-250 g (PGI strain) and bred in our animal colony were employed for the present study. They were maintained on rat chow (Hindustan Lever Products) and had free access to water.

Chronic diazepam treatment schedule

Diazepam was administered every day in a dose of 20 mg/kg, intraperitoneally (ip) for a period of three weeks. Control animals received a
proportional volume of 1% carboxymethylcellulose as vehicle. The injections were made daily between 8 a.m. and 9 a.m. The body weights of animals were recorded on 0, 10 and 21 day of drug treatment.

**Drug treatment schedule**

On completion of chronic treatment with diazepam for 3 weeks, the rats were divided into different groups for drug treatment and each group consisted of a minimum of five animals.

The diazepam-withdrawn animals in different groups received two doses of clonidine (50 µg/kg and 100 µg/kg), guanfacine (106 µg/kg and 500 µg/kg) or B-HT 920 (106 µg/kg and 500 µg/kg). The 106 µg/kg dose of guanfacine and B-HT 920 was equivalent to 100 µg/kg clonidine on a molar basis. Each rat received two injections at 12-hr intervals, the first injection being made at 8:00 a.m. No drug treatment was given to animals in the control group after diazepam withdrawal.

In another set of experiments, chronically diazepam-treated rats were pretreated with yohimbine (1.5 mg/kg) 30 min prior to each clonidine (100 µg/kg) injection. The effect of yohimbine (1.5 mg/kg) alone was also studied in chronically diazepam-treated rats.
A group of rats receiving only the diazepam injection vehicle (1% carboxymethylcellulose) for 3 weeks served as untreated control. The effects of clonidine, guanfacine and B-HT 920 alone were also studied in non-diazepam-treated animals in order to assess any effect of these agents on normal behaviour.

**Measurement of withdrawal symptoms**

The withdrawal hyperactivity symptoms were measured using a photoactometer (Techno) for measuring horizontal activity (Dews, 1953) and activity wheel (Techno/modified) for measuring vertical activity of the animals. The activity wheel consisted of a rotating cage wheel with a diameter of 28 cm and 14 cm wide. Animals were placed individually inside the cage wheel and as the animal climbed the curvature (vertical activity), the wheel moved down. The movements/rotation of the cage wheel were counted by a photoelectric device. All the animals in various treatment groups were made accustomed to both the apparatus by taking four trial readings in four days before the actual measurement.
In the drug-treated groups, the activity was recorded daily 2.5 hr after the first dose of the drug. A cut-off time of 10 min was kept constant for both the types of hyperactivity studies. The withdrawal hyperactivity was studied for four days after the termination of chronic diazepam treatment.

Drugs

Diazepam (Ranbaxy, New Delhi) was uniformly dispersed in 1% carboxymethylcellulose, clonidine (Boehringer, Ingelheim, FRG) guanfacine (Sandoz, Switzerland), B-HT 920 (Boehringer, Ingelheim, FRG) and yohimbine (E.Merck) were dissolved in distilled water. Injection volume 5 ml/kg was kept constant. All drugs were administered intraperitoneally.

Statistical analysis

Statistical analysis was done using Student's t test.
AUTONOMIC HYPERACTIVITY ON DIAZEPAM WITHDRAWAL IN RATS

**Animals and techniques**

Male albino Wistar rats (200-250 g) bred in our animal colony maintained on rat chow (Hindustan Lever) and free access to water, were used in the present study.

**Diazepam treatment**

Diazepam (20 mg/kg, ip) was administered every day at 9.00 a.m. for three weeks. Control rats received a proportional volume of 1% carboxymethylcellulose as vehicle. On completion of 3 weeks of diazepam treatment, the drug was withdrawn and the animals were divided into different groups consisting of minimum of six animals in each.

One group served as withdrawal control, the second group was treated with clonidine (100 μg/kg, ip, twice daily at 12 hr interval). Clonidine treatment was started on the day of diazepam withdrawal, and continued till the third day of withdrawal.
The other groups were used for brain monoamine estimation and for the measurement of heart rate and locomotion.

**Brain amines**

On third day of diazepam withdrawal, the animals were sacrificed using Harvard decapitator and whole brain was removed and homogenized in 10 volume of cold butanol for estimation of noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). These substances in the brain tissues were estimated spectrophotofluorometrically (Hitachi 650-40) by the method of Cox and Perhach (1973).

**Materials** Analytical grade reagents and glass distilled water were used.

**Standard** 100 µg/ml of l-noradrenaline hydrochloride (Sigma), dopamine hydrochloride (BDH), serotonin creatinine sulfate (Sigma) and 5-hydroxy-3-indoleacetic acid (Sigma) were prepared with 0.01 N hydrochloric acid (HCl, Analar BDH).

**Acidified butanol** Acidified butanol was prepared by adding 0.85 ml of concentrated HCl to one litre of n-butyl alcohol.
0.1 M EDTA-1 M Sodium acetate (pH 7.0)

0.1 M EDTA (disodium ethylenediamine tetraacetate dehydrate) (37.2 g) was dissolved in 1M sodium acetate and made up to a volume of 1 litre. The pH was adjusted to 6.7-7.0 by addition of sodium hydroxide.

0.1 N Iodine 1.27 g iodine was dissolved in 100 ml of absolute ethanol.

Alkaline sulfite One ml of sodium sulfite solution (2.5 g of anhydrous salt in 10 ml of water) was diluted with 9 ml of 5 N sodium hydroxide (NaOH) just before experimental use.

0.35 M Borate buffer (pH 11) Boric acid (31.4 g) was dissolved in 1 litre of distilled water and 55 ml 10 N sodium hydroxide added. This was then saturated with n-butanol and sodium chloride and adjusted to pH 11 if necessary.

Alumina About 200 g of chromatographic grade alumina (BDH) was boiled in 1 litre 1 N HCl for 30 min then washed with 20 x 100 ml water, or until the pH of the washing had risen to between 4 and 5. Finally, it was left to dry overnight at room temperature and then heated at 200°C for 2 hr.
**0.033 N Sodium bicarbonate** It was prepared in glass distilled water by dissolving 0.277 g of sodium bicarbonate in 100 ml of water.

**0-Phthalaldehyde** 10 mg/100 ml of 0-phthalaldehyde was prepared in 10 N HCl.

**Assay procedures** Brain samples were homogenized in 10 ml of ice cold acidified butanol with a mechanical mixer (Silverson Machines Ltd., U.K.). Brain samples weighing less than 280 mg, were made up to 280 mg with distilled water before homogenization in 2.8 ml of butanol. The homogenates were centrifuged for 5 min at 800 g. A 2.5 ml portion of the supernatant fluid was transferred to a 13 ml glass-stoppered centrifuge tube containing 2.5 ml of distilled water and 5 ml of heptane. The tubes were shaken for 5 min and centrifuged at 800 g for 5 min; 6 ml of the organic phase was transferred to a clean 13 ml glass-stoppered centrifuge tube and refrigerated for determination of 5-HIAA. Also 2.5 ml of the aqueous phase was transferred to screw-capped test tube containing 200 mg of alumina, to it 1 ml of 2.0 m sodium acetate was added and the tubes were gently shaken for 10 min and centrifuged for 5 min.
at 800 g. From the aqueous phase, 3.0 ml was transferred to clean screw-capped test tube and refrigerated for subsequent determination of 5-HT. The remaining aqueous phase was aspirated from the alumina. The alumina was then washed by shaking with 2.0 ml of distilled water for 5 min and centrifuged for 5 min at 800 g. The aqueous phase was discarded and 2.0 ml of 0.1 N acetic acid was added to the alumina. The tubes were gently shaken for 10 min and centrifuged at 800 g for 5 min; 10 ml of aqueous phase was transferred to a small test tube (13 x 75 mm) for fluorescent assay of NA and DA.

For the estimation of NA and DA, the pH of acetic acid extract was adjusted to 4. Then, to this solution 0.2 ml of 0.1 N EDTA reagent was added and the mixture was adjusted to a pH of 6.5. Then 0.1 ml of 0.1 N iodine was added to oxidise the catecholamines. After exactly 2 min, the oxidation was stopped by addition of 0.2 ml of alkaline sulfite and exactly 2 min later the pH of the solution was adjusted to 5.4 by addition of 0.2 ml of 5 N acetic acid. To assay NA, the fluorescence was read after 60 min (activation at 385 nm and emission at 485 nm).
or to shorten the 60 min interval the mixture was heated in boiling water for exactly 2 min.

To assay DA, the solution was heated at a temperature of boiling water for 5 min and the fluorescence was read at activation 320 nm and emission 370 nm.

To extract 5-HIAA, 0.4 ml of 0.033 N sodium bicarbonate was added to the tube containing the organic phase obtained from the initial transfer step. The tubes were gently shaken for 5 min and centrifuged for 5 min at 800 g; 0.3 ml of the aqueous phase was transferred to a test tube containing 0.7 ml of ophthaldehyde reagent. The tube contents were mixed and then heated in a boiling water for 10 min. After cooling in running tap water, the fluorescence was read with the activation and emission wavelengths set at 360 and 470 nm, respectively.

To extract 5-HT, 3.0 g of NaCl, 1.0 ml of 0.35 M borate buffer (pH 11.0) and 6.0 ml of n-butanol were added to the aqueous phase transferred after initial shake with alumina. The tubes were shaken for 10 min and centrifuged for 5 min at 800 g; 5.0 ml of the organic phase was transferred to a centrifuge
tube containing 0.5 ml of 0.1 N HCl and 6 ml of heptane. The tubes were shaken for 5 min and centrifuged for 5 min at 800 g; 0.4 ml of the aqueous phase was transferred to a test tube and 0.6 ml of the ophthaldialdehyde was added. The tube contents were mixed and then heated in boiling water for 10 min. After cooling, fluorescence was read with the activation and emission wavelength set at 360 and 470 nm, respectively.

Reagent blank: A reagent blank was carried out with the same procedure except using 2.5 ml acidified butanol in place of tissue homogenate.

For calculation, tissue blank was subtracted from the standard and reagent blank was subtracted from the test samples. For finding out the concentration in test samples, standard curve was plotted with 100, 200, 400, 600, 800 ng of NA, DA, 5-HT and 5-HIAA, respectively (Fig. 9 and Fig. 10). The concentration of test samples was calculated by linear regression analysis. The following different groups were processed (1) animals on third day of chronic diazepam withdrawal, (2) animals on third day of clonidine therapy after the termination of chronic diazepam,
Fig. 9
Standard curve of noradrenaline (NA)  

Standard curve of dopamine (DA)
Standard curve of 5-hydroxytryptamine (5-HT) and Standard curve of 5-hydroxyindole acetic acid (5-HIAA)

Fig. 10

Fluorometric Reading vs. Conc. (ng/2.5 ml) 5-Hydroxytryptamine 5-Hydroxyindole Acetic Acid
(3) animals on chronic diazepam treatment, and (4) control animals which received neither diazepam nor any drug treatment.

**Hyperlocomotion and heart rate**

The change in basal heart rate as an index of hyperfunction of adrenergic system was recorded using an electrocardiograph. The study was done on third day of chronic diazepam withdrawal. The locomotor activity of the animals was measured using a photometer (Dews, 1953) as described in the first section. The locomotor activity of individual animal was studied for a period of 10 min.

**Drugs**

Diazepam (Ranbaxy, New Delhi) was uniformly suspended in 1% carboxymethylcellulose. Clonidine (Boehringer Ingelheim, New York) was dissolved in distilled water. Noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) (Sigma, USA) were used as standards. All reagents used in the assay were of recommended analytical grade.
**Statistical analysis**

Statistical analysis was done using Student's t test.

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

**Animals and technique**

Albino rats of Wistar strain of either sex (80-100 g) from our breeding colony were used for the present study. Previous reports from our laboratory have shown that young rats were more prone to develop stress ulcers. They were deprived of food and allowed free access to water 24 hr before the start of the experiments. Animals were kept in cages provided with meshed aluminium base to avoid coprophagy. Selection of various doses of drugs was based on preliminary studies conducted in our laboratory.
Immobilization stress-induced ulcers

Twenty-four hr fasted young rats were restrained by placing them on their back on the operation table and tying the four limbs to the table for 3 hr. (Kunchandy et al., 1985). Animals were sacrificed at the end of 3 hr and stomachs examined for spot ulcers and apparent bleeding. Drug treatment was made at the beginning of the stress. All antagonists were given 15 min prior to agonists.

Ulcer index

The stomach was cut along the greater curvature and washed gently under running tap water. It was then scored for ulcer index as follows: 0 = normal coloured stomach; 0.5 = red colouration; 1 = spot ulcers; 1.5 = for each haemorrhagic streaks; 2 = ulcers more than 2 and less than 5, and 3 = ulcers 5 or more than 5. Mean ulcer score for each animal was calculated and expressed as ulcer index.

Drugs

Diazepam (Ranbaxy, New Delhi), clonazepam (Roche, Hertfordshire), chlordiazepoxide (Roche,
Bombay), Ro 5-4864 (Hoffmann-la Roche, Nutley, NJ), and Ro 15-1788 (F. Hoffman-la Roche, Basel) were uniformly dispersed in 1% CMC with a drop of Tween 80. Phenytoin sodium (Parke Davis, Bombay) was dissolved in distilled water. Muscimol (Sigma, St. Louis, MO), bicuculline (Sigma, St. Louis, MO) and baclofen (Ciba Geigy, Basel) were dissolved in distilled water with few drops of 0.01 N hydrochloride acid. All injections were made intraperitoneally in a constant volume of 0.5 ml/100 g body weight.

**Statistical analysis**

Student's t test was used for the analysis of data.

**NALOXONE- SENSITIVE AND GABA_\text{A} RECEPTOR MEDIATED ANALGESIC RESPONSE OF BENZODIAZEPINES IN MICE**

**Animals and technique**

Albino mice of either sex weighing 20-30 g (PGI, Chandigarh) were employed. They were maintained on chow (Lipton India Ltd.) and had free access to water. The analgesic effect due to endogenous opioid
release was quantified by the tail-flick method using INCO analgesiometer.

Measurement of analgesia

The analgesic effect (pain threshold to radiant heat) was measured by the tail flick method using an analgesiometer (D'Amour and Smith, 1941; Kulkarni, 1980). Keeping a constant cut-off time of 10 sec (4A current), basal reaction time to radiant heat was established before drug administration and subsequent readings were taken for 3 hrs, at intervals as described earlier (D'Amour and Smith, 1941). The antagonists were given 15 min prior to the agonists.

Drugs

Diazepam (Ranbaxy, New Delhi), clonazepam (Roche, Hertfordshire, UK), chlordiazepoxide (Roche, Bombay), Ro 5-4864 (Hoffmann-La Roche, Nutley USA), Ro 15-1788 (F. Hoffmann-La Roche, Switzerland), nitrazepam (Cipla, Bombay) and lorazepam (Cipla, Bombay) were uniformly suspended in 1% carboxymethylcellulose with a drop of Tween 80. Phenytoin sodium (Parke-Davis, Bombay), sodium pentobarbitone (Loba, Bombay) and naloxone hydrochloride (Endo,
New York) were dissolved in distilled water. Bicuculline (Sigma, USA), muscimol (Sigma, USA) and baclofen (Ciba Geigy, Switzerland) were dissolved in water with a few drops of 0.1 N hydrochloric acid. All drugs solutions were administered intraperitoneally at a constant volume of 0.1 ml/10 g body weight.

Statistical analysis

Statistical analysis was done using Student's t test.

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

Animals and technique

Adult Wistar mice of either sex (20-30 g) bred in the CAH animal colony (Panjab University, Chandigarh) were employed. For ICV studies, Wistar rats (200-250 g) were used.

An intraperitoneal dose of 60 mg/kg of Ro 5-4864 produced convulsions in all the animals. All experiments were conducted between 13.00 and 16.00 hr, since a variation in the convulsive effect was seen when experiments were carried out at different times of the day. After injecting a convulsant dose
of Ro 5-4864, mice were transferred to an observation cage and were observed for the next 30 min. The latency of the onset of the first myoclonic jerk and the first full body convulsion were noted as described by Dunwiddie and Worth (1982). Death or recovery from the convulsions was recorded.

**ICV administration**

Wistar rats (200-250 g) of either sex were anaesthetised with ether. A polyethylene cannula was chronically implanted in the left lateral ventricle for intracerebroventricular drug administration (Noble et al., 1967). After a recovery period of 5-7 days, animals were used for experimentation. Drugs were administered ICV by a Hamilton microsyringe in volumes not exceeding 20 μl. Control rats received an equal amount of saline.

**Drug treatment**

All agonists were administered 15 min prior to the convulsant Ro 5-4864, except phenytoin which was given 30 min prior to Ro 5-4864. Similarly, antagonists were given 10-15 min prior to the agonists. In the ICV studies alpha₂ adrenoceptor agonists were given
5 min before a convulsant dose of Ro 5-4864.

Drugs

Ro 5-4864 (chlorodiazepam, Hoffmann-La Roche, Nutley), Ro 15-1788 (flumazepil, F. Hoffmann-La Roche, Basel) and clonazepam (Roche Products, Hertfordshire) were uniformly suspended in water with a few drops of Tween 80. Diazepam (Ranbaxy, New Delhi) was suspended in 1% carboxymethylcellulose with a drop of Tween 80. Clonidine (Boehringer, Ingelheim, FRG), guanfacine (Sandoz, Switzerland), B-HT 920 (6-Allyl-2-amino-5,6,7,8-tetrahydro-5H-pyrrolo (1,2a)imidazole derivative, (Imperial Chemical Industries, Cheshire, UK), phenytoin sodium (Parke Davis, Bombay), yohimbine hydrochloride (E. Merck), idazoxan (Reckitt & Colman, Hull), pentobarbitone sodium (Loba, Bombay) and CL 218 872 (3-methyl-6-(3-(trifluoromethyl)-phenyl)-1,2,4-triazolo (4,3b) pyridazine, Lederle, New York) were dissolved in distilled water. Injection volume 10 mL/kg was kept constant for mice. All drugs were administered intraperitoneally.
Statistical analysis

Statistical analysis was done using Student's t test and analysis of variance (ANOVA) employing 95% confidence level.

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

Animals and technique

Wistar mice of either sex (25-30 g) from CAH animal colony (Panjab University, Chandigarh) were employed for the present study. They were subjected to hypoxic stress by putting them individually in a glass container of 370 ml capacity. The containers were tightly closed with greased glass lids to ensure airtightness. Under these circumstances, the animals showed convulsions and mortality due to hypoxia. The latency for convolution and death were noted as described by Bhargava (1986). Control studies were run in parallel. Drugs were given 15 min prior to the stress.

For icv studies, Wistar rats of either sex (220-250 g) from CAH animal colony (Panjab University, Chandigarh) were used. Drugs were injected into the
right lateral cerebral ventricle through a permanent indwelling cannula fixed on the skull as described by Noble et al. (1967). Injections were made with the help of a Hamilton micro syringe. Injections volume of 20 μl/rat was kept constant. For stress effects, rats were immediately subjected to hypoxic stress by placing them in an airtight glass container of 2.5 litre capacity. The latency for death was noted.

Drugs

Clonidine (Boehringer, Ingelheim, FRG), guanfacine (Sandoz, Switzerland), B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d) azepine dihydrochloride, Boehringer, Ingelheim, FRG), ICI 106270 (6-aryl 2,3,6,7-tetrahydro-5H-pyrrolo (1,2-a)-imidazole derivative, Imperial Chemical Industries, U.K.), yohimbine hydrochloride (E.Merck), CL 218872 (3-methyl-6-(3-(trifluoromethyl)-phenyl)-1,2,4-triazole (4,36) pyridazine, Lederle, New York) were dissolved in distilled water. Diazepam (Ranbaxy, New Delhi) and Ro 5-4864 (p-chlorodiazepam, Hoffman-La Roche, Nutley) were uniformly suspended in distilled water with a few drops of Tween 80. Injection volume of 10 ml/kg was kept constant. Antagonists were given 15 min prior to the agonists. All drugs were injected intraperitoneally.
Statistical analysis

ANOVA test with 99% confidence level and Student's t test were employed.

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

Animals and technique

Wistar mice of either sex (25-30 g) from the CAH animal colony (Panjab University, Chandigarh) were used. Food and water were withdrawn one hour prior to the experiments.

Convulsions were induced by DMCM (25 mg/kg, ip) or Ro 5-4864 (60 mg/kg, ip). These doses were selected on the basis of preliminary study or based on the earlier report from our laboratory. The latencies of jerk and full body convulsions were noted as described by Dunwiddie and Worth (1982). Doses of clonidine, guanfacine, B-HT 920 and ICI 106270 were given 15 min. prior to the convulsant. Antagonists were given 10-15 min prior to the agonists.
Drugs

DMCM (Schering, Berlin), Ro 5-4864 (Hoffmann-La Roche, Nutley), Ro 15-1788 (F. Hoffmann-La Roche, Basel), clonidine (Boehringer, Ingelheim, FRG), guanfacine (Sandoz, Switzerland), B-HT 920 (Boehringer, Ingelheim, FRG), ICI 106 270 (Imperial chemical Industries, U.K.) and idazoxan (Reckitt & Colman, Hull) were used in the present study. DMCM, Ro 5-4864 and Ro 15-1788 were uniformly dispersed in distilled water with a few drops of Tween 80. Injection volume of 10 ml/kg was kept constant. All drugs were administered intraperitoneally.

Statistical analysis

Statistical analysis was done using Student's t test.

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

Animals and technique

Experiments were performed on Wistar mice of either sex (20-30 g) obtained from the CAH animal colony (Panjab University, Chandigarh). Food and
water were withdrawn about one hour before the experiment.

**Pentylentetrazole-induced seizures**

Convulsions were induced in mice with pentylentetrazole (100 mg/kg, ip). The animals were then kept in groups of three in glass chamber and the latency for the first myoclonic jerk or whole body convulsions were noted as described by Dunwiddie and Worth (1982).

Adenosine and diazepam were given 5 min and 30 min, respectively, prior to the convulsant dose of PTZ. All drugs were given intraperitoneally.

**Drugs**

Adenosine (Loba, Bombay) was uniformly dispersed in water with a few drops of Tween 80. Diazepam (Ranbaxy, New Delhi) was dispersed in 1% CMC with few drops of Tween 80. Pentylentetrazole (Sigma, U.S.A.) and dipyridamole (German remedies, Bombay) were dissolved in water. Injection volume of 10 ml/kg was kept constant. Control animals were treated with respective vehicles.
APPARENT pA₂ ESTIMATION OF BENZODIAZEPINE RECEPTOR ANTAGONISTS

Animals and technique

Albino Wistar rats of either sex (150-200 g) from our breeding colony were employed. The experiments were conducted between 9.00 and 12.00 hr at room temperature. Food and water were withheld during the experimentation.

Convulsions were induced in rats by injecting 80 mg/kg of pentylenetetrazole (PTZ). The severity of convulsion was assessed by the following scale devised according to the nature of CNS excitation and convulsion produced by PTZ.

0 = normal
1 = straub tail
2 = body jerks
3 = whole body convulsions
4 = death
A cumulative score was calculated for each animal. A set of control (vehicle treated) experiments were carried out in parallel giving PTZ (80 mg/kg) to these animals. The percentage of protection by various doses of diazepam was determined before and after the treatment with different doses of BZ antagonists. The antagonists were given ten min prior to the agonist and each group consisted of a minimum of five animals.

The in vivo equivalent of $pA_2$ (apparent $pA_2$) values were determined for Ro 15-1788-diazepam pair and CGS 8216-diazepam pair according to Hayashi and Takemori, (1971) and Fürst et al., (1982). The percentage protective dose response curves were determined for diazepam alone and in combination with three doses (0.5 mg/kg, 1 mg/kg and 2 mg/kg) of Ro 15-1788 and CGS 8216. The dose ratios (DR) were calculated and log doses in moles/kg of Ro 15-1788 and CGS 8216 were plotted against log (DR-1) as described by Hayashi and Takemori (1971).

**Analysis**

ED$_{50}$ values were calculated by plotting the log doses of diazepam versus the percentage protective
effect of diazepam against PTZ seizures. The dose response curve of diazepam with three different doses of BZ antagonists was determined and the dose ratio calculated as:

$$DR = \frac{ED_{50} \text{ with the antagonist}}{ED_{50} \text{ without antagonist}}$$

The molecular weight of Ro 15-1788 and CGS 8216 were taken as 303.3 and 261.29, respectively. The pA\textsubscript{2} values were estimated by plotting log (DR-1) (as ordinate) versus log moles/kg of antagonist. The negative value of the point where the line intersected the abscissa at the zero level of the ordinate is taken as the pA\textsubscript{2} value from the above Schild plot.

**Drugs**

Diazepam (Ranbaxy, New Delhi), CGS 8216 (Ciba Geigy, Switzerland) and Ro 15-1788 (F. Hoffmann-La Roche, Switzerland) were uniformly dispersed in 1% carboxymethylcellulose with a drop of Tween 80. Pentylenetetrazole (Sigma, USA) was dissolved in distilled water. All drugs were administered intraperitoneally at constant volume of 0.5 ml/100 g body weight. The control animals received only the vehicle.
Summary of various drugs employed in the present study.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Nature</th>
<th>Dose (mg/kg, ip)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>BZ receptor agonist</td>
<td>5-20</td>
<td>Ranbaxy, New Delhi</td>
</tr>
<tr>
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<td>Central BZ receptor agonist</td>
<td>3</td>
<td>Roche Products, Hertfordshire.</td>
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<td>BZ receptor agonist</td>
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<td>Cipla, Bombay</td>
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<td>Cipla, Bombay</td>
</tr>
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<td>CL 218 872</td>
<td>BZ receptor agonist</td>
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<td>Lederle, New York</td>
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<td>45,60</td>
<td>Hoffmann- La Roche, Nutley</td>
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<tr>
<td>Compound</td>
<td>Effect</td>
<td>Concentration</td>
<td>Source</td>
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<td>Phenytoin sodium</td>
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<td>BZ receptor antagonist</td>
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<td>F. Hoffmann-La Roche Basel</td>
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<td>Pentobarbital sodium</td>
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<td>5-30</td>
<td>Loba Chemie, Wein Fischamend</td>
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<td>Clonidine</td>
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<td>Alpha&lt;sub&gt;2&lt;/sub&gt; adrenoceptor agonist</td>
<td>0.1-1</td>
<td>Sandoz, Switzerland</td>
</tr>
<tr>
<td>B-HT 920 Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Alpha&lt;sub&gt;2&lt;/sub&gt; adrenoceptor agonist</td>
<td>0.1-1</td>
<td>Boehringer, Ingelheim</td>
</tr>
<tr>
<td>Compound</td>
<td>Action</td>
<td>Dose (μg/icv)</td>
<td>Supplier</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------------</td>
<td>---------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>ICI 106 270</td>
<td>$\alpha_2$ adrenoceptor agonist</td>
<td>0.1-1,10</td>
<td>Imperial Chemicals, Cheshire</td>
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<tr>
<td>Yohimbine HCl</td>
<td>$\alpha_2$ adrenoceptor antagonist</td>
<td>1.5-5</td>
<td>E. Merk</td>
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<tr>
<td>Idazoxan</td>
<td>$\alpha_2$ adrenoceptor antagonist</td>
<td>1</td>
<td>Reckitt &amp; Colman, Hull.</td>
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<tr>
<td>Adenosine</td>
<td>$P_1$-purinoceptor agonist</td>
<td>25-100</td>
<td>Loba-Chemie Wien Fischamend.</td>
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<tr>
<td>Naloxone HCl</td>
<td>Opiate receptor antagonist</td>
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<td>Endo, New York</td>
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<tr>
<td>DMCM</td>
<td>BZ-Inverse agonist</td>
<td>25</td>
<td>Schering, Berlin</td>
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<tr>
<td>Dipyridamole</td>
<td>Inhibitor of adenosine uptake 1 and metabolism</td>
<td>5</td>
<td>German Remedies</td>
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</tbody>
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