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Differences in the Duration of Sedative and Anxiolytic Effects of Desmethyldiazepam in Two Outbred Wistar Strains

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VAN DER LAAN, J. W., S. F. DE BOER, J. VAN DER GUGTEN AND G. DE GROOT. *Differences in the duration of sedative and anxiolytic effects of desmethyldiazepam in two outbred Wistar strains.* PHARMACOL BIOCHEM BEHAV **39**(1) 149–153, 1991.—Different sensitivities to benzodiazepines have been described for various strains of both rats and mice suggesting that variations in biological features of the animals are responsible for these differences. Since all reports concern inbred strains, we studied two outbred Wistar strains which are used routinely in several research disciplines. The pharmacodynamics of desmethyldiazepam (DMD), the main active metabolite of diazepam in man, were compared for male rats of the Riv:TOX strain (from the National Institute of Public Health and Environmental Protection) and the Crl:(WI)BR strain. The duration of sedative action of DMD after oral administration, as derived from suppression of the nocturnal locomotor activity, was longer in the Riv:TOX strain than in the Crl:(WI)BR strain. Accordingly, suppression of novelty-induced corticosterone release as an index of anxiolytic action was observed 11 hours after DMD administration in Riv:TOX rats but not in Crl:(WI)BR rats. At that time, serum DMD concentration was shown to be higher in the Riv:TOX strain than in the Crl:(WI)BR strain. The data are discussed in relation with possible metabolic differences between the two strains.

Desmethyldiazepam Rat strains Novelty stress Corticosterone response

BENZODIAZEPINES are widely studied in animals for their anxiolytic, hypnotic, anticonvulsant and muscle-relaxant effects. Animal behaviour studies with the benzodiazepines have been hampered by large variations in drug effects, e.g., on rotarod performance (7) and on conflict behaviour (3). Recently, various mouse strains have been described which differ in their sensitivity to benzodiazepines, suggesting that genetic properties of the animals are responsible for the variations in anticonvulsant and muscle-relaxant response (4, 6, 28). Also various rat strains were found to differ in the "emotionality response" (open field defecation) and in the amount of benzodiazepine receptors in brain, e.g., the Maudsley reactive strain (20,25) and the Roman rat strains (22). All these strains are inbred and are in use only with respect to specific research questions.

The present paper, in contrast, describes differences between two outbred Wistar strains which are used routinely in various disciplines. A systematic comparison of the pharmacodynamic aspects of desmethyldiazepam (DMD) has been made. Special attention has been given to the duration of sedation (suppression of nocturnal locomotor activity) and stress-decreasing properties (inhibition of the corticosterone increase in response to a novel environment). We have tested whether the observed differences in pharmacological effectiveness can be ascribed to a difference in the same direction in the serum concentrations of desmethyldiazepam.

METHOD

Animals

Male albino rats from two sources were used. Riv:TOX (M) rats were provided by The National Institute of Public Health and Environmental Protection. Crl:(WI)BR rats were obtained

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from Charles River WIGA, Sulzfeld, FRG. After arrival in the animal housing the rats were kept for 4–5 days in wire mesh cages with water and Hope Farm food pellets freely available. A light/dark schedule of 12 hours (lights on at 07.00) was maintained. The temperature was 22–24°C.

Drugs

Desmethyldiazepam was received from Will-Pharma, Zwanenburg, The Netherlands. Prazepam (used as internal standard in the HPLC-desmethyldiazepam assay) was a gift of Parke Davis, Amsterdam. Desmethyldiazepam was suspended in saline by use of Tween 80 in an administration volume of 5 ml/kg.

Locomotor Activity and Food Intake

The animals (body weight for both strains 150–200 g at the test day) used for the activity meter experiments were initially housed two in a wire-mesh cage for 10–14 days (lights on from 05.00 to 17.00 h); they received SSP-tox food, a semisynthetic diet (Trouw BV, Putten, The Netherlands). Subsequently the animals were placed individually during one night in PVC cages ($40 \times 25 \times 30$ cm) of the activity meter with one glass wall and sawdust bedding. SSP-Tox food was present in a tin in a corner of the cage and a bottle of water was attached through a hole in the wall. DMD was administered orally at 16.30 h. Food intake was measured by weighing the tins the next morning, 16–17 hours after treatment. Spillage was not taken into account, since the spilled food mixed with the sawdust. However, after careful observation, the spillage was estimated to be usually not higher than 1 g. Animals were used only once. The activity was measured as the number of interruptions of two crossed light beams directed at two infrared photocells (Sick FR2-12), and counted by two 8-channel counters each consisting of a front-end and an Arcom ARC-41 single board computer. These two counters were coupled with an Apple IIe microcomputer. The data were then in turn transmitted to an Altos computer, which under a Xenix operating system further carried out the analysis.

Corticosterone Response to Novelty Stress

The animals (body weight for both strains 275–325 g at the first test day) used for plasma corticosterone (CS) determinations were housed individually in clear Plexiglas cages on woodshavings with free access to food and water under conditions of constant temperature (21 ± 2°C) and a fixed 12-h light/dark photoperiod (lights on from 08.00 to 20.00 h). Under ether anesthesia animals were provided with a silicon cannula (i.d. 0.5 mm; o.d. 1.0 mm) into the entrance of the right atrium (vena cava) via an external jugular venotomy according to the techniques basically described by Steffens (24). This method allows frequent withdrawal of small amounts of blood without disturbing the animals either behaviorally or physiologically (24,27). For at least one week, animals were allowed to recover from surgery and to habituate to the sampling procedure.

In the morning on the experimental day, 11 hours after oral administration of DMD (200 mg/kg) or vehicle, a blood sample was taken in the home cage of the undisturbed rat, in order to measure basal level of plasma CS. Subsequently, the rat was picked up and placed for 30 min in another cage of the same size containing clean sawdust without food and water (novel environmental stress). Blood samples for determination of stress-induced CS elevation were taken at $t = 15$ and 30 min after transfer. Blood was replaced by transfusion of heparinized blood through the cannula after each blood sampling. From all animals, blood was sampled under both drug and vehicle conditions according to a balanced repeated measures within-subject design with an intertest interval of 4 days.

Blood samples were collected in chilled (0°C) centrifuge tubes containing heparin as anticoagulant (25 units per ml). After centrifugation, supernatants were removed and stored at −30°C for the CS assay. Plasma CS concentrations were determined in duplicate according to a competitive protein binding method (18). For determination of DMD levels (see below) blood was obtained upon decapitation 11 hours after oral drug administration in a separate experiment. Subsequently, serum was prepared and stored in polypropylene cups at −20°C.

Serum Desmethyldiazepam Concentration

Quantitation of DMD was carried out either by reversed-phase HPLC analysis (2) or by radioreceptor assay (RRA). For HPLC, desmethyldiazepam was extracted from serum by a solid phase extraction procedure. Prazepam was used as an internal standard. Detection of the drugs was by ultraviolet detection at 225 nm.

Radioreceptor assay was carried out according to Hunt et al. (8), modified with respect to radioligand and receptor preparation (17) and adapted to small serum samples. Desmethyldiazepam was extracted from 20 μl samples in 175 μl ethylacetate. Extract fractions were evaporated and incubated in duplicate with 900 μl bovine frontal cortex homogenate and 25 nCi [methyl-^{3}H]flunitrazepam (60 Ci/mmol; NEN Chemicals) at 4°C for 60 min. Unbound benzodiazepine was removed by rapid filtration through Whatman GF-B filters. DMD standards were used to calculate desmethyldiazepam equivalent levels in serum. A serum DMD concentration of 1.23 ± 1.3 μmol/l resulted in 50% inhibition of specific radioligand binding.

Statistical Analysis

Data for locomotor activity and food intake were evaluated by ANOVA. Post hoc analysis was carried out with the nonparametric Mann-Whitney U-test (23). Corticosterone response data and serum DMD values were analysed using ANOVA followed by a post hoc *t*-test to detect the source of detected significance. The criterion of significance was set at $p<0.05$.

For the serum DMD values a one-tailed test was used since it was expected that the DMD serum concentration was lower in that strain in which no or hardly any effect could be seen.

RESULTS

Locomotor Activity and Food Intake

DMD was given orally around 30 minutes before darkness. To assess the approximate duration of action of DMD, the night was divided in three periods of four hours. The data depicted in Fig. 1 indicate that the activities of the vehicle-treated groups of both strains did not differ. In the course of the night a tendency towards an increase in activity was present.

In the first period, DMD (100 mg/kg PO) already induced a maximal suppression of locomotor activity in the Riv:TOX rat by 50–60% of the initial activity. Also in the Crl:(WI)BR rat, the maximal effect in the first period was already obtained after the 100 mg/kg dose. In the second period, the decrease was dose-dependent in both rat strains. In the Crl:(WI)BR strain, the effect of the highest dose was not as strong as found in the first period. In the third period, DMD has lost its effectiveness in suppressing locomotor activity in this strain at all doses tested.

Locomotor activity data were evaluated by a 2-way ANOVA

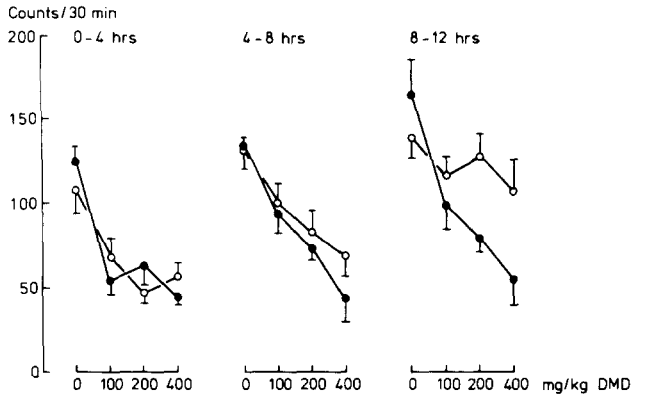

FIG. 1. Effects of desmethyldiazepam (DMD) on nocturnal locomotor activity in two rat strains. Rats were placed individually in activity cages with two infrared beams placed crosswise. At 16.30 p.m. DMD was administered orally in the doses indicated. Lights were off from 17.00–05.00. The experiments were carried out separately for each strain. Activity was measured at hourly intervals and summated for 4-hour periods. Data are given as means ± SEM. *$p<0.05$ Mann-Whitney U-test. (●)Riv:TOX (n = 6); (○)Crl:(WI)BR (n = 8).

with strain as between-subjects factor (2 levels), and dose as within subjects factor (4 levels) for each period. For the third period the effects of strain and dose as well as the interaction between the two were significant [strain: $F(1,48)=4.55$, $p=0.04$; dose: $F(3,48)=7.59$, $p<0.001$; strain and dose interaction: $F(3,48)=2.73$, $p=0.05$].

Analysis of food intake data (Fig. 2) revealed a significant effect of strain, $F(1,56)=5.18$, $p<0.025$, being high in the Crl:(WI)BR rat. Also a dose-dependent effect of DMD was found to be present, $F(3,56)=3.47$, $p=<0.025$, resulting in a strain × dose interaction, $F(3,56)=4.86$, $p<0.005$. The source of this dose-strain interaction appeared to be especially the 400 mg/kg dose in the Riv:TOX rat.

Corticosterone Response to Novelty Stress

A property of the benzodiazepines related to their anxiolytic activity is their ability to increase basal CS secretion and/or to reduce the rise in CS that results from the stress of a novel environment. The effectiveness of this property was measured in the two rat strains after oral application of DMD.

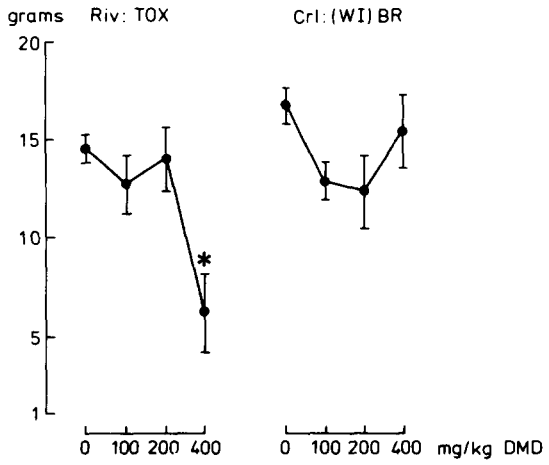

FIG. 2. Effect of desmethyldiazepam (DMD) in two rat strains on the food intake during the night in the activity meter. At 16.30 DMD was administered orally in the doses indicated. The amount of food intake was measured at 09.00 the next morning. Data are given as means ± SEM. *$p<0.05$, t-test after ANOVA.

Figure 3a shows the effect of DMD (200 mg/kg PO) on basal CS release and on the novelty stress-induced CS elevation in both strains of rats. Data were evaluated first by a 3-way ANOVA with strain as between-subjects factor (2 levels), treatment as within-subjects factor one (2 levels) and sampling time as within-subjects factor two (3 levels). ANOVA revealed significant main effects of strain, $F(1,10)=9.87$, $p=0.010$, and sampling time, $F(2,9)=69.78$, $p<0.0001$, as well as a significant interaction between treatment and sampling time, $F(2,9)=13.11$, $p=0.0025$, and an overall significant strain × treatment × sampling time interaction, $F(2,9)=11.31$, $p=0.0038$. After vehicle treatment, the stressful situation produced clearly an increase in plasma CS. However, this rise in CS concentration after placing the rat in the novel cage appeared to be significantly higher in the Riv:TOX strain than in the Crl:(WI)BR strain. DMD was shown to increase the basal level of corticosterone at time 0 in the Riv:TOX rat, but diminished the strong rise due to changing the environment of the rat. In the Crl:(WI)BR rat, no effect of DMD either on basal level or on the stress response was observed.

In Fig. 3b, drug effects on novelty-induced CS responses, quantified by computing the integrated areas under the response-time curves, are depicted more clearly. DMD strongly prevented the CS increase in the Riv:TOX strain but not in the Crl:(WI)BR strain indicating that DMD has a shorter action in the latter strain.

Table 1 shows the DMD serum concentrations 11 hours after the oral administration of 200 mg DMD in Tween 80/saline suspension. The mean DMD concentration was higher in the Riv:TOX rat ($p<0.05$, t-test, one-tailed). A correlation was present between the HPLC and the RRA serum DMD values (Pearson: r = .9801). The mean values obtained with both assays are similar.

DISCUSSION

The present data show the existence of a strain difference in the duration of pharmacodynamic effects of desmethyldiazepam.

The duration of action of a compound is to some extent related to the elimination half-life, however, there is no direct correlation due to tissue distribution. The elimination half-life of desmethyldiazepam in rats is known to be short. For Charles River CD-1 rats a value of 65.4 min has been reported (5) and for Sprague-Dawley rats a value of 60 min (19). Recently, we described for Riv:TOX rats an elimination half-life of DMD after intravenous administration of 70.3 min and for Crl:(WI)BR rats a value of 33.8 min (Van der Laan et al., submitted). The biochemical background for this difference was suggested to be related to a difference in the capacity of liver metabolism.

The shorter elimination half-life of DMD in the Crl:(WI)BR rat, compared to the Riv:TOX rat, is in agreement with the shorter action of DMD on nocturnal locomotor activity, as described in the present paper. Eight hours after oral administration no effect of DMD was found in the Crl:(WI)BR rat, while DMD was still active in the Riv:TOX rat.

The duration of action of DMD on nocturnal locomotor activity (i.e., in the order of magnitude of 8–10 hours), however, is much longer than the elimination half-life mentioned above. Because DMD was given orally as a suspension and not as a solution in a relatively high dose, its absorption was probably prolonged (19) due to a kind of gastrointestinal depot releasing DMD slowly. Elimination data for benzodiazepines in suspen-

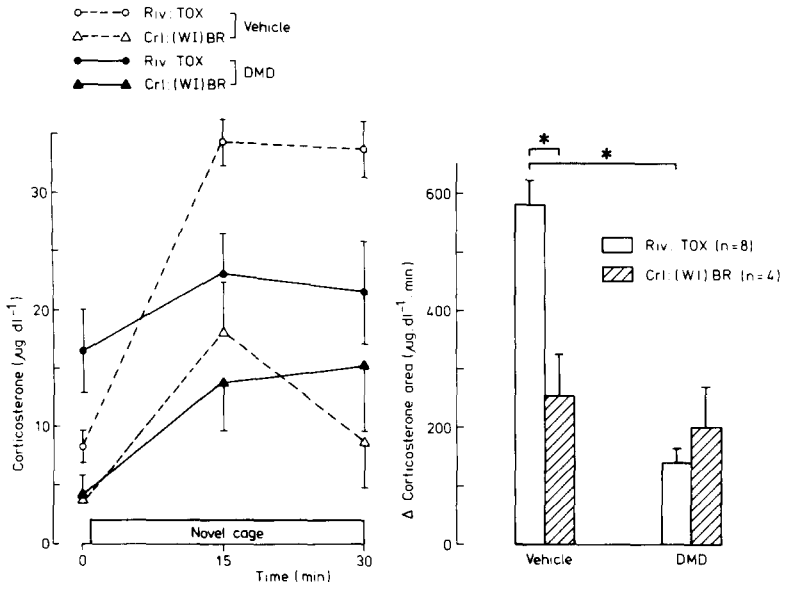

FIG. 3. Comparison of the effects of desmethyldiazepam (DMD) in two rat strains on basal level of plasma CS and its elevation induced by novelty stress. DMD (200 mg/kg) or vehicle was administered orally 11 hours before the animals were subjected to the novel environment stress. Data are expressed as means ± SEM of plasma CS levels (A) and as mean integrated responses of plasma CS (B).

sion after oral administration are lacking. Nonetheless, the use of such high dosages is not unusual in studies on benzodiazepine dependence, since it is known that inducing benzodiazepine dependency orally necessitates the use of such high dosages (14,21).

The low food intake in the Riv:TOX rat after DMD 400 mg/kg may be indicative for a toxic effect at this dose. Benzodiazepines are known to act as appetite stimulants (1) especially at low doses. The dosage used in the present experiment appeared to be too high to obtain such a stimulatory effect causing on the contrary an inhibitory effect possibly due to excessive sedation.

The strain difference in the duration of action of DMD is confirmed by the difference in effect on the CS levels under basal and novelty stress conditions. Eleven hours after administration of DMD two effects were present in the Riv:TOX rat which were absent in the Crl:(WI)BR rat. Firstly, DMD elevated the basal CS concentration in plasma. Secondly, DMD was found to decrease the stress-induced rise in plasma CS.

TABLE 1

CONCENTRATIONS OF DESMETHYLDIAZEPAM IN SERUM (µmol/l; MEAN ± STANDARD ERROR OF THE MEAN) 11 H AFTER ORAL ADMINISTRATION OF 200 mg/kg DESMETHYLDIAZEPAM MEASURED EITHER BY HPLC OR BY RRA

	DMD Serum Concentration in µmol/l	
Strain	HPLC	RRA
Riv:TOX n=8	5.593 ± 1.915	5.164 ± 1.541
Crl:(WI)BR n=8	2.371 ± 0.522*	2.451 ± 0.523*

*$p<0.05$, t-test (one-tailed).

The enhancement of basal CS concentration is in general agreement with several other studies showing a dose-dependent increase in basal plasma CS release following acute benzodiazepine administration (12, 15, 16, 26). This ability of benzodiazepines to increase basal CS secretion has been related to the behaviorally depressant action of these drugs (16,26). However, the exact site and mechanism of action of this property has not been elucidated yet.

The second effect of DMD on CS was a reduction of the rise induced by the exposure to a novel environment. Such an inhibition, which was only present in the Riv:TOX strain, is in agreement with the data reported by Lahti and Barshun (10) and Le Fur et al. (11). The ability of BDZ to prevent stress-induced increases in CS release has been related to the anxiolytic property of these drugs (10, 11, 16, 26). This effect appears to be mediated via a central site since diazepam was unable to block the rise of plasma CS produced by adrenocorticotrophic hormone (11) and intracerebroventricularly administered chlordiazepoxide exerted effects similar to those observed following systemic administration (12).

The present explanation for the mechanism of action of DMD on corticosterone stress response in the Riv:TOX implicates that 11 hours after administration of DMD (200 mg/kg PO) this drug is still active in this strain, while it is not active in the Crl:(WI)BR strain confirming the effects on nocturnal locomotor activity. This reflects probably a slower elimination of DMD in the Riv:TOX rat in comparison with the Crl:(WI)BR rat as is indicated also by the higher DMD serum concentration in the Riv:TOX rat 11 hours after oral administration (Table 1).

It might be argued that the HPLC assay of DMD leads to an underestimation of the benzodiazepine activity in serum since it is known that in many species metabolism of DMD results in the formation of the active metabolite oxazepam. However, it

has been reported that in rats hardly any oxazepam is detectable after DMD administration (13). Moreover, the similar values found after HPLC and RRA in the present study (Table 1) indicate that DMD was the only active benzodiazepine compound in serum.

It must be considered that the benzodiazepine inhibition of stress-induced CS secretion might be related to the effects of DMD on the basal CS level. A long-lasting enhancement of CS concentrations may lead to a higher feedback via the glucocorticoid receptor at the hypophyseal level which can lead to a lower pituitary-adrenocortical response under stress-conditions (9). Therefore, in the Riv:TOX strain, the CS response to stress might have been diminished because of the elevated basal concentrations, while in the Crl:(WI)BR strain there was no reduction of the CS response to stress because no effect was found on the basal level.

In conclusion, both the longer-lasting effect of DMD on nocturnal locomotor activity and on the CS rise after exposure to novelty stress in the Riv:TOX rat as opposed to the Crl:(WI)BR strain indicate that DMD has a longer action in the Riv:TOX strain. The higher serum concentration of DMD found in the latter strain can be suggested to be related to a longer elimination half-life of DMD in this particular strain.

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REFERENCES

1. Cooper, S. J. Benzodiazepine-opiate antagonist interactions and reward processes: implications for drug dependency. Neuropharmacology 22:535–538; 1983.
2. De Groot, G.; Grotenhuis-Mullenders, A. M. L. J. Combined bonded phase/liquid-liquid extraction as a rapid specific sample treatment technique for the determination of benzodiazepines in plasma by RP-HPLC. In: Maes, R. A. A., ed. Topics in forensic and analytical toxicology. Amsterdam: Elsevier; 1984:95–103.
3. Duka, T.; Cumin, R.; Haefely, W.; Herz, A. Naloxone blocks the effect of diazepam and meprobamate on conflict behaviour in rats. Pharmacol. Biochem. Behav. 15:115–117; 1981.
4. File, S. E.; Greenblatt, D. J.; Martin, I. L.; Brown, C. Long-lasting anticonvulsant effects of diazepam in different mouse strains: correlations with brain concentrations and receptor occupancy. Psychopharmacology (Berlin) 86:137–141; 1985.
5. Friedman, H.; Abernethy, D. R.; Greenblatt, D. J.; Shader, R. I. The pharmacokinetics of diazepam and desmethyldiazepam in rat brain and plasma. Psychopharmacology (Berlin) 88:267–270; 1986.
6. Gallaher, E. J.; Hollister, L. E.; Gionet, S. E.; Crabbe, J. C. Mouse lines selected for genetic difference in diazepam sensitivity. Psychopharmacology (Berlin) 93:25–30; 1987.
7. Henauer, S. A.; Gallaher, E. J.; Hollister, L. E. Long-lasting single-dose tolerance to neurologic deficits induced by diazepam. Psychopharmacology (Berlin) 82:161–163; 1984.
8. Hunt, P.; Husson, J. M.; Raynaud, J. P. A radioreceptor assay for benzodiazepines. J. Pharm. Pharmacol. 31:448–451; 1979.
9. Keller-Wood, M. E.; Dallman, M. F. Corticosteroid inhibition of ACTH secretion. Endocr. Rev. 5:1–24; 1984.
10. Lahti, R. A.; Barshun, C. The effect of minor tranquilizers on stress-induced increases in rat plasma corticosteroids. Psychopharmacologia 35:215–220; 1974.
11. Le Fur, G.; Guilloux, F.; Mitrani, N.; Mizoule, J.; Uzan, A. Relationships between plasma corticosteroids and benzodiazepines in stress. J. Pharmacol. Exp. Ther. 211:305–308; 1979.
12. McElroy, J. F.; Miller, J. M.; Meyer, J. S. Comparison of the effects of chlordiazepoxide and CL 218,872 on serum corticosterone concentrations in rats. Psychopharmacology (Berlin) 91:467–472; 1987.
13. Marcucci, F.; Mussini, E.; Fanelli, R.; Garattini, S. Species differences in diazepam metabolism—I. Metabolism of diazepam metabolites. Biochem. Pharmacol. 19:1847–1851; 1970.
14. Martin, W. R.; McNicholas, L. F.; Cherian, S. Diazepam and pentobarbital dependence in the rat. Life Sci. 31:721–730; 1982.
15. Matheson, S. K.; Sage, D.; White, D.; Diton, V.; Gipson, D. A comparison of the effects of buspirone and diazepam on plasma corticosterone levels in rat. Neuropharmacology 27:823–830; 1988.
16. Mormede, P.; Dantzer, R.; Perio, A. Relationship of the effects of the benzodiazepine derivative clorazepate on corticosterone secretion with its behavioral actions. Antagonism by RO 15-1788. Pharmacol. Biochem. Behav. 21:823–843; 1984.
17. Muller, W. E. The benzodiazepine receptor. Cambridge: Cambridge University Press; 1987:145–151.
18. Murphy, B. E. P. Some studies of the protein-binding of steroids and their application to the routine micro- and ultramicro measurement of various steroids in body fluids by competitive protein-binding radio assay. J. Clin. Endocrinol. Metab. 27:973–983; 1967.
19. Pacifici, G. M.; Cuoci, L.; Placidi, G. F. Disposition, distribution, and liver first-pass effect of pinazepam in the rat. Drug Metab. Dispos. 10:180–182; 1982.
20. Robertson, H. A.; Martin, I. L.; Candy, J. M. Differences in benzodiazepine receptor binding in Maudsley Reactive and Maudsley Non-reactive rats. Eur. J. Pharmacol. 50:455–462; 1978.
21. Ryan, G. P.; Boisse, N. R. Experimental induction of benzodiazepine tolerance and dependence. J. Pharmacol. Exp. Ther. 226: 100–107; 1983.
22. Shephard, R. A.; Nielsen, E. B; Broadhurst, P. L. Sex and strain differences in benzodiazepine receptor binding in Roman rat strains. Eur. J. Pharmacol. 77:327–330; 1982.
23. Siegel, S. Non-parametric statistics for the behavioural sciences. Kogakusha: McGraw Hill; 1956.
24. Steffens, A. B. A method for frequent sampling blood and continuous infusion of fluids in the rat without disturbing the animal. Physiol. Behav. 4:833–836; 1969.
25. Tamborska, E.; Insel, T.; Marangos, P. J. "Peripheral" and "central" type benzodiazepine receptors in Maudsley rats. Eur. J. Pharmacol. 126:281–287; 1986.
26. Torrellas, A.; Guatra, C.; Borrell, J. Effects of acute and prolonged administration of chlordiazepoxide upon the pituitary-adrenal activity and brain catecholamines in sound stress and unstressed rats. Neurosciences 5:2289–2295; 1980.
27. Wiersma, J.; Kastelijn, J. A. A chronic technique for high frequency blood sampling/transfusion in the freely behaving rat which does not affect prolactin and corticosterone secretion. J. Endocrinol. 107:285–291; 1985.
28. Wilks, L.; File, S. E.; Martin, I. L. Evidence of strain differences in GABA-benzodiazepine coupling. Psychopharmacology (Berlin) 93:127–132; 1987.