Pharmacology Biochemistry and Behavior, Vol. 65, No. 3, pp. 539–546, 2000

0091-3057/00/$–see front matter

PII S0091-3057(99)00190-2

Diazepam Has No Beneficial Effects on Stress-Induced Behavioural and Endocrine Changes in Male Tree Shrews

MARJA VAN KAMPEN,* ULRICH SCHMITT,† CHRISTOPH HIEMKE† AND EBERHARD FUCHS*

*Division of Neurobiology, German Primate Centre, 37077 Göttingen, Germany; and †Department of Psychiatry, University of Mainz, 55131, Mainz, Germany

Received 18 May 1999; Revised 23 July 1999; Accepted 12 August 1999

VAN KAMPEN, M., U. SCHMITT, C. HIEMKE AND E. FUCHS. *Diazepam has no beneficial effects on stress-induced behavioural and endocrine changes in male tree shrews.* PHARMACOL BIOCHEM BEHAV **65**(3) 539–546, 2000.— The present study evaluated the effect of subchronic oral treatment of psychosocially stressed male tree shrews with diazepam on locomotor activity, marking behavior, avoidance behavior, and urinary cortisol and noradrenaline. To mimic a realistic situation of anxiolytic intervention, the treatment started 14 days after the beginning of psychosocial stress; at that time, the stress-induced behavioral and endocrine alterations had been established. The drug (5 mg/kg/day) was administered orally in the morning, while the psychosocial stress continued during the whole treatment period; the therapeutic action of diazepam treatment was followed across 7 days. Twenty-four hours after the last application serum concentrations of diazepam and its major metabolites were determined via HPLC. The results revealed concentrations of 7 ng/ml for diazepam, 106 ng/ml for nordiazepam, 22 ng/ml for temazepam, and 30 ng/ml for oxazepam. Treatment of subordinate animals with diazepam did not reveal a beneficial effect to any of the parameters studied. This contrasts to earlier findings showing that the behavioral and neuroendocrine alterations produced by this stress paradigm are sensitive to chronic treatment with the tricyclic antidepressant clomipramine. The present results support the view that in male tree shrews the state induced by psychosocial stress might be more depression related than anxiety related. © 2000 Elsevier Science Inc.

Anxiety Depression Cortisol Noradrenaline Avoidance behavior Marking behavior Locomotor activity Animal models Stress

DURING recent years, many experimental paradigms have been developed to investigate laboratory animals in relation to their responses to challenging or stressful situations (42,56). These preclinical models are of particular importance and interest, because in humans, stress is thought to initiate or exacerbate a number of psychiatric disorders such as generalized anxiety, posttraumatic stress syndrome, or major depression (21,48,55). Consequently, animal models have been extremely useful in elaborating and detecting the effects of psychotropic drugs. However, in some of the models the stressors used bear little or no relationship to the biology of the species investigated because they involve noxious stimuli or perturbations of the physical environment such as electric foot shock, forced swimming, physical restraint, water and food deprivation, or cold exposure. Moreover, animals, predominantly rodents, are investigated during their sleeping phase, picked up and removed from their home cage, and transferred to an unfamiliar environment such as an open field, a swimming tank, or a shuttle box. These settings clearly contrast to the situation in humans where most common stressors are of a more psychological type. To improve our knowledge of the causal mechanisms of stress-related disorders, and to bridge the gap between experimental models and the situation in humans, we need in animal studies more naturalistic models with psychological types of stressors. Evidence has accumulated in recent years that the psychosocial stress model in male tree shrews (*Tupaia belangeri*) may represent a promising paradigm. Phylogenetically tree shrews are re-

Requests for reprints should be addressed to Eberhard Fuchs, Division of Neurobiology; German Primate Centre, Kellnerweg 4; 37077 Göttingen, Germany.

garded as an intermediate between insectivores and primates (35). In their natural habitats in Southeast Asia, adult male tree shrews display an intense territoriality (25) that can be used to establish a naturally occurring challenge situation under experimental control in the laboratory. Coexistence of two males in visual and olfactory contact leads to a stable dominant–subordinate relationship, with subordinates showing distinct stress-induced behavioral, physiological, and central nervous alterations. Their marking behavior and locomotor activity is clearly suppressed (2), their circadian rhythm is profoundly disturbed (17,47), and their sleeping pattern is characterized by an increasing number of early waking episodes in the second half of the night (2). The reduction of body weight from the moment of subjugation onwards is due to a diminished food and water intake and to a significantly elevated metabolic rate (15,23). Analysis of endocrine parameters in subordinates revealed constantly increased concentration of the adrenocortical hormone cortisol and enlarged adrenal glands (14,39,53) being indicative for a hyperactivity of the hypothalamo–pituitary–adrenal (HPA) system (14). Moreover, subordinate animals have reduced activities of the gonads (11) and consequently, low plasma testosterone levels (54). In the hippocampus, we recently demonstrated down-regulation of glucocorticoid and corticotropin-releasing hormone (CRH) receptors (13,24).

In humans, depressive disorders are a collection of symptoms that occur together with a sufficient frequency to constitute a recognizable clinical condition. Patients suffering from major depression frequently show a psychomotor retardation, phase shift in circadian activity patterns, early morning awakenings, appetite disturbances and weight loss, hyperactivity of the HPA system, and loss of libido (7). Phenomenologically, these symptoms in depressed patients are comparable to the biobehavioral responses observed in subordinate tree shrews. To elucidate whether the tree shrew model, besides its obvious "face validity" for depression, also has a "predictive validity" (56,57), we treated subordinate animals with the antidepressant clomipramine (16). The drug had a time-dependent restorative influence on marking behavior, locomotor activity, avoidance behavior, as well as on urinary cortisol excretion. It, thus, appeared that the clomipramine treatment counteracts the behavioral and endocrine effects of chronic psychosocial stress and the time course of recovery corresponds closely to that observed when treating depressed patients.

As demonstrated by studies in humans (32) and animals (37,49) clomipramine also has, besides its well-documented antidepressant effects, anxiolytic properties. To further validate the tree shrew model and to investigate whether the observed stress-induced responses are anxiety related and could be reversed by anxiolytic treatment, we investigated the action of the prototypic benzodiazepine receptor agonist diazepam on locomotor activity, marking behavior, avoidance behavior, and urinary free cortisol and noradrenaline in subordinate animals. To mimic a realistic situation of anxiolytic intervention, the treatment started after the stress-induced behavioral and endocrine alterations had been established. Furthermore, the drug was administered orally, while the psychosocial stress continued. In view of clinical patterns, it was of obvious importance to examine the effects of treatment following subchronic, rather than acute, administration. Thus, the potential therapeutic action of diazepam was followed across 7 days. To assess the role of pharmacokinetics, serum concentrations of diazepam and its major metabolites were determined at the end of the treatment.

METHOD

Animals and Housing

Adult male tree shrews (*Tupaia belangeri*) were obtained from the breeding colony at the German Primate Centre, Göttingen, Germany. The animals were housed singly on a regular day/night cycle (lights on from 0800 to 2000 h; for housing conditions see (12). All animal experimentation was conducted in accordance with the European Communities Council Directive of November 24th, 1986 (86/EEC), and was approved by the Government of Lower Saxony, Germany.

Drug Application

Tree shrews received diazepam (Valiquid®, Hoffmann–LaRoche AG, Grenzlach-Wyhler, Germany) via a bulb-headed cannula into the bucal cavity, and were allowed to swallow the solution. This per os route of administration was chosen to minimize uncontrollable stress effects associated with common methods of drug administration in experimental animals such as IP or SC injection. Furthermore, oral application is the most common route of administration of anxiolytic medication in humans.

In a pilot study, we determined the diazepam dosage necessary to reach a plasma concentration in tree strews similar to that known to be herapeutically effective in humans. For 7 consecutive days animals received 5 mg/kg diazepam dissolved in 6% ethanol and distilled water in the morning. This dose is anxiolytically effective in rats when given acutely (43). The drug solutions were freshly prepared before every application. Twenty-four hours after the last drug application, blood was collected by puncturing the tail's venous plexus.

Determination of Drug Concentrations in Serum Samples

Diazepam and its pharmacologically active metabolites were analyzed in serum by solid phase extraction and subsequent high-performance liquid chromatography (HPLC). For extraction from plasma a solid phase extraction procedure was performed by loading 450 μl serum on the C_8-coated extraction columns (ICT, Hengoed, UK) using an automatic sample processor (ASPEC, Gilson, Villiers le Bel, France). Prior to sampling, loading columns were sequentially conditioned with 1.0 ml methanol and 1.0 ml water. Loaded columns were washed with 1.0 ml of 50% methanol. Elution was performed using 1.0 ml methanol. Eluates were evaporated at 40°C in an evaporator system (Bachhofer, München, Germany). For injection into the HPLC system the dried analytes were redissolved in 0.5 ml HPLC eluent.

The HPLC system was composed of an HPLC-compact pump (Model 2200, Bischoff, Leonberg, Germany) used with a flow rate of 1.5 ml/min, a Shimadzu SPD 10-A UV detector set at 242 nm, and a PROMIS type II autosampler (Spark, Emmen, The Netherlands) with a 100-μl sample loop. A 125 × 4-mm 5 μm particle size RP-Select B analytical column (MZ-Analysentechnik, Mainz, Germany) was used for chromatographic separation of diazepam and its major metabolites—nordiazepam, oxazepam, and temazepam. The analytical eluent consisted of 60% methanol, and 0.01 mol/1 acetic buffer with pH 4.66. The chromatograms were recorded and integrated by a Kontron PC Integration pack version 3.90 (Kontron Instruments, Milan, Italy). Baseline separation was obtained with <10 min with respective retention times of 4.2 min for diazepam, 3.45 min for nordiazepam, 2.85 min for temazepam, and 2.4 min for oxazepam. The limit of detection based on the signal-to-noise ratio of at least 3:1 was 5 ng/ml for all sub-

stances, with a sensitivity of 10.7% (range, 6.0–16.6%). Linearity was found up to concentrations of 400 ng/ml.

Experimental Procedure

During a 10-day control period (days 1–10) individually housed male tree shrews ($n = 10$), were weighed daily between 0745 and 0800 h; the basal activities of the pituitary–adrenocortical axis and of the sympathetic system were determined by measuring cortisol and nonadrenaline in morning urine that was collected after a slight massage of the hypogastrium.

In the first experimental group (stress + diazepam group; $n = 5$), the induction of a psychosocial conflict was carried out according to our standard procedure [for details, see (16).] After the control period, each male of the stress + diazepam group was introduced into the territory of a socially experienced male. This resulted in an active competition for control over the territory. After establishment of a stable dominant/subordinate relationship, the two males were separated by a transparent wire mesh barrier. The barrier was removed every day for 1–1.5 h between 0830 and 1000 h, allowing physical contact between the two males. After 14 days of psychosocial conflict (days 11–24), subordinate animals received diazepam (5 mg/kg) orally every day in the morning. For the next 7 days (days 25–31), the subordinate animals remained in the psychosocial conflict situation, and were treated daily with diazepam.

After the control period, animals of the second group (no stress + diazepam group; $n = 5$) remained singly housed in their home cages for another 14 days (days 11–24). After this period, they received diazepam (5 mg/kg) orally in the morning for 7 days (days 25–31). Daily all animals were weighed, and morning urine samples were collected. Twenty-four hours after the last drug application, blood was collected from all animals by puncturing the tail's venous plexi.

Monitoring and Analysis of Behavior

The behavior from each experimental animal was videotaped daily between 1845 and 1915 h. By using a behavior sampling procedure in a 15-min interval three types of behavior were quantified from the tapes.

Locomotor activity was measured using the Insight Software program from OCTEC Ltd. (Bracknell, UK). For this purpose the cage was divided on the monitor in six quadrants of equal size (w:25 cm × h:40 cm; head–tail length of the animals: approx. 30 cm). Similar to quantification of motor activity in an open field, movements were counted as one event whenever an animal moved from one quadrant to an adjacent one. Marking behavior was quantified by measuring the total time the animal spent with different forms of scent marking such as marking with the abdominal gland, marking with the sternal gland, and urinary marking (2,34,46). According to Aue (2), subordinate tree shrews tend to avoid visual contact with the dominant conspecific. To quantify this avoidance behavior, we measured the time every animal spent in the sleeping box. All behavior analyses were performed with the Observer 3.1 software (Noldus Technology, Wageningen, The Netherlands).

Analysis of Urine Samples

Urinary free cortisol was determined with a radioimmunoassay and urinary noradrenaline was quantified by HPLC [for details, see (16)]. To correct for physiological dilution of urine, the resulting concentrations were related to creatinine concentrations.

Statistical Analysis

The statistical analysis of the data was performed using the GB-Stat 5.3 software (Dynamic Microsystems, Silver Spring, MD). To avoid interferences between interindividual pretest values, all results were transformed into percent values by relating them to the individual's mean value of the control period. The data of every experimental group were divided in three blocks: control (10 days; days 1–10), stress/no stress (14 days; days 11–24), and stress + diazepam/no stress + diazepam (7 days; days 25–31). For significance testing, the means of the three treatment blocks were compared. To test for significant differences of means in one experimental group we used the Friedman test with the Wilcoxon signed-rank test as the post hoc test. For the statistical analysis of plasma concentrations of diazepam and its metabolites, the Mann–Whitney–*U*-test was used. Statistical significance was sent at $p < 0.05$. Data are expressed as means ± SEM.

RESULTS

Diazepam Concentrations in Serum

After a 7-day period of treatment with the anxiolytic, blood samples were taken from the animals of the stress + diazepam group and no stress + diazepam group for monitoring drug concentrations in serum. In these samples, diazepam and its major metabolites (nordiazepam, oxazepam, and temazepam) were detected with high interindividual variability (Table 1). Compared to the no stress + diazepam group the stress + diazepam group showed significantly higher levels ($p < 0.05$) of the metabolite nordiazepam and a trend ($p = 0.07$) for a higher concentration of oxazepam 24 h after the last application.

Body Weight

A decrease in body weight is an important indicator by which tree shrews may be classified as being subordinate (14,54). In the stress + diazepam group, the subordinate animals displayed a significant reduction in body weight during the first two weeks of psychosocial conflict (Fig. 1a; stress period; $p < 0.05$) and the decline continued during the treatment period (stress + diazepam treatment; $p < 0.05$). This

TABLE 1

SERUM CONCENTRATIONS OF DIAZEPAM AND ITS MAJOR METABOLITES AFTER A TREATMENT PERIOD OF 7 DAYS (5 mg/kg/DAY; PO) AND 24 h AFTER THE LAST ADMINISTRATION

	No Stress + Diazepam ($n = 5$)	Stress + Diazepam ($n = 5$)
Diazepam	4 ± 3	7 ± 5 NS
Nordiazepam	17 ± 7	106 ± 58 $p < 0.05$
Temazepam	4 ± 4	22 ± 12 NS
Oxazepam	14 ± 6	30 ± 8 $p = 0.07$

Statistical differences between the no stress + diazepam and the stress + diazepam group are indicated. Data (ng/ml) are given as means ± SEM.

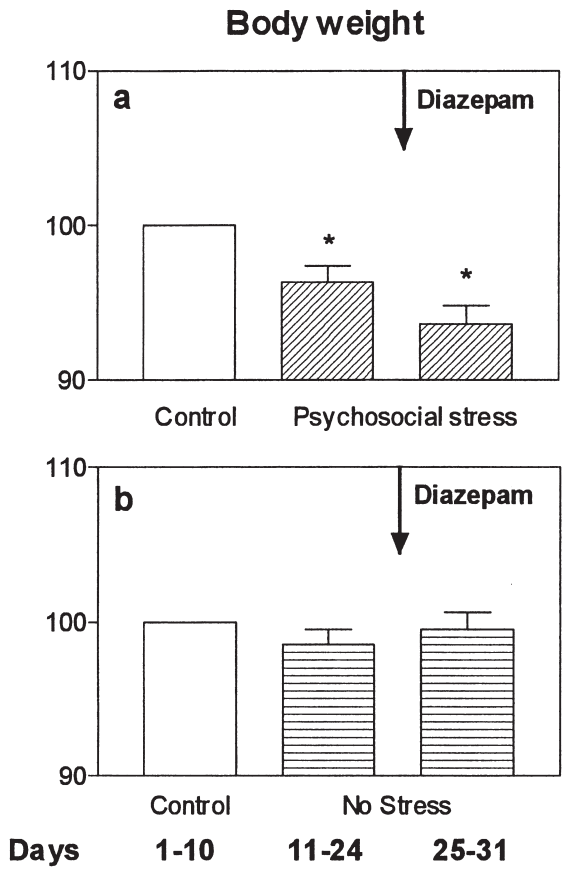

FIG. 1. Effect of psychosocial stress and diazepam treatment on body weight in male tree shrews. (a) Stress + diazepam group; (b) no stress + diazepam group. The anxiolytic was applied orally (5 mg/kg) starting on day 20 of the experiment (indicated by the arrow) and the daily diazepam treatment was continued until day 27. Data were transformed into percent values by relating them to the individual's mean value of the control period and are given as means ± SD. Significant differences: * $p \leq 0.05$

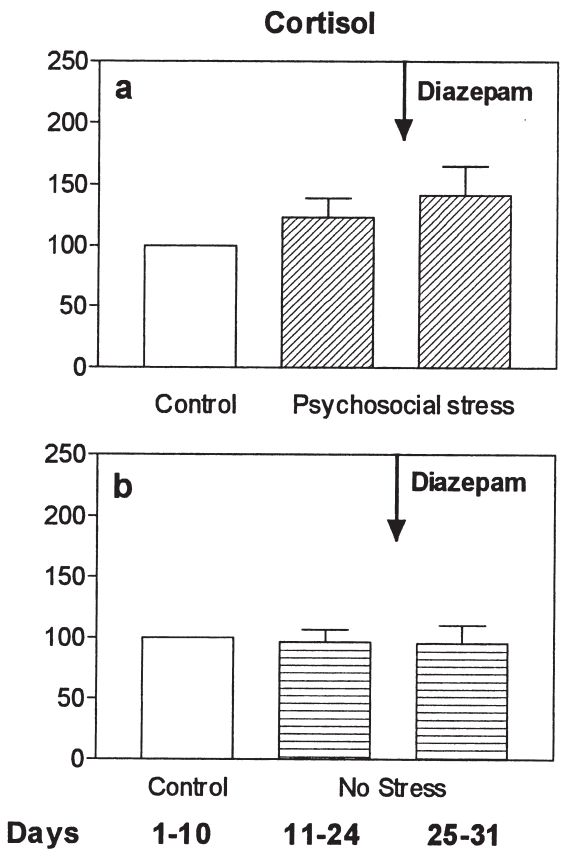

FIG. 2. Effect of psychosocial stress and diazepam treatment on urinary free cortisol in male tree shrews. (a) Stress + diazepam group; (b) no stress + diazepam group. For details see Fig. 1.

finding contrasts to the no stress + diazepam group showing no treatment effects on body weight (Fig. 1b).

Urinary Parameters

The effect of psychosocial stress in subordinate tree shrews is demonstrated by an activation of the HPA axis as indicated by the elevation of urinary cortisol excretion during stress exposure (Fig. 2a). Despite the daily treatment of subordinate animals with diazepam (5 mg/kg), the cortisol excretion still increased. No effects on urinary cortisol excretion were observed in animals from the no stress + diazepam group (Fig. 2b).

Besides the activation of the HPA axis, chronic psychosocial stress also resulted in an activation of the neurosympathetic tone, as demonstrated by the significant increase of urinary noradrenaline after 2 weeks of social encounters (Fig. 3a; $p < 0.05$). Compared to the control period, stress and daily anxiolytic treatment resulted in a marked increase in the urinary excretion of the catecholamine ($p < 0.05$). Treatment of no-stress animals with diazepam for 7 days, however, had no significant effects on the urinary noradrenaline concentration compared to the control situation (Fig. 3b).

Behavioral Parameters

The effects of psychosocial conflict and of oral diazepam treatment on marking behavior are summarized in Fig. 4. Marking behavior was reduced for more than 50% within the first 2 weeks of social encounters ($p < 0.05$). Daily administration of diazepam had no restorative effect. In contrast, during the 7-day stress and treatment period marking behavior decreased to nearly 10% of the control level (Fig. 4a). Despite a tendency to higher individual variation, no significant diazepam-related effects on marking behavior were observed in the control group (no stress + diazepam; Fig. 4b).

Qualitatively, a similar pattern of stress and diazepam effect was observed in locomotor activity. In subordinate animals, locomotor activity significantly declined during the first 2 weeks of social stress and during stress and drug application period (Fig. 5a; stress + diazepam; $p < 0.05$). The treatment of control animals with diazepam had no influence on their locomotor activity (Fig. 5b).

In general, subordinate tree shrews also tend to avoid the dominant conspecific in situations when the two animals are separated by a wire mesh barrier. This avoidance behavior and the effects of diazepam treatment is summarized in Fig. 6. During stress exposure there is a strong tendency for an increase in the time the subordinates spent in the sleeping box quadrant (Fig. 6a; $p = 0.07$). Diazepam treatment for 7 days, however, resulted in a tendency to reduce avoidance behav-

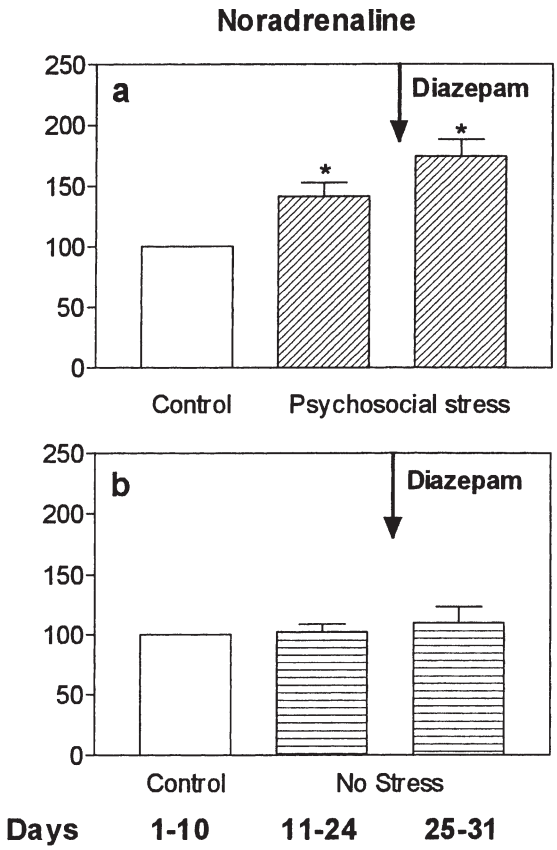

FIG. 3. Effect of psychosocial stress and diazepam treatment on urinary noradrenaline in male tree shrews. (a) Stress + diazepam group; (b) no stress + diazepam group. For details see Fig. 1.

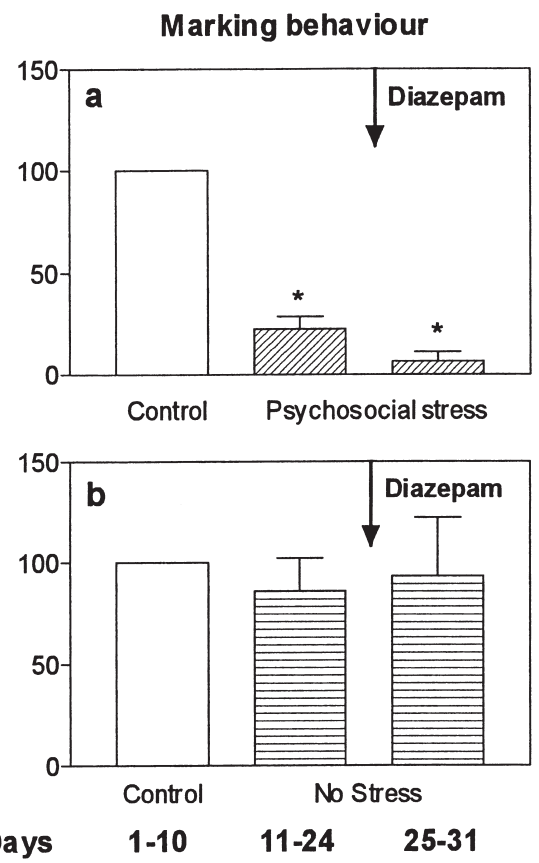

FIG. 4. Effect of psychosocial stress and diazepam treatment on marking behavior in male tree shrews. (a) Stress + diazepam group; (b) no stress + diazepam group. For details see Fig. 1.

ior. The treatment of control animals (no stress + diazepam) had no effect on their avoidance behavior (Fig. 6b).

DISCUSSION

In these experiments we have investigated the effect of subchronic administration of the traditional anxiolytic drug diazepam on behavior and neuroendocrine functions in psychosocially stressed male tree shrews. The primary aim was to investigate whether treatment with diazepam (5 mg/kg/day) could reverse the biobehavioral changes resulting from psychosocial conflict. In male tree shrews, the coexistence of two males leads to a stable dominant/subordinate relationship, representing a stressful situation for the subordinate individuals (2). The distinct physiological and behavioral alterations observed in subordinates result exclusively from the cognitive interpretation of the situation and the continuous presence of the dominant conspecific (39,54). In line with these earlier findings, the present study revealed for subordinate animals a significant reduction in body weight, a clear activation of the HPA axis, and the sympatho–adrenal system indicated by the pronounced increases in urinary cortisol and noradrenaline excretion, and distinct changes in behavioral variables such as marking behavior, locomotor activity, and avoidance behavior. Treatment of subordinate animals with diazepam for 7 days did not reveal a consistent beneficial effect to any of the parameters studied. This contrasts to earlier findings showing that the behavioral and neuroendocrine alterations produced by this stress paradigm are sensitive to chronic treatment with the tricyclic antidepressant clomipramine (16).

Stimulation of the HPA axis as indicated by raised plasma glucocorticoid hormone concentrations and activation of the neurosympathetic and adrenomedullary branches of the sympatho–adrenal system — reflected by increases in circulating noradrenaline — are hallmarks of the neuroendocrine responses to emotional stressors in mammals (19). During stress conditions brain benzodiazepine receptors appear to participate in the physiological regulation of adrenocortical and neurosympathetic activity (4). Numerous reports have demonstrated that benzodiazepine receptor ligands with anxiolytic actions like diazepam can prevent or oppose the stress-induced activation of HPA and sympathetic–adrenomedullary system (5,9,10,28,29,31,36,38,52).

Consequently, it would be expected that diazepam reduces the activity of stress-activated neuroendocrine systems that was not observed in the present study. One explanation may arise from earlier findings demonstrating that stress exposure may alter the responses to benzodiazepines over prolonged periods of time. In rats, exposure to a brief stressful event up to at least 1 month earlier completely prevented the effect of diazepam on plasma corticosterone (1). Furthermore, several laboratories have reported that the effects of benzodiazepines on HPA axis activity in intact animals are not simple. Small doses do not alter basal activity of the axis (30,33), whereas

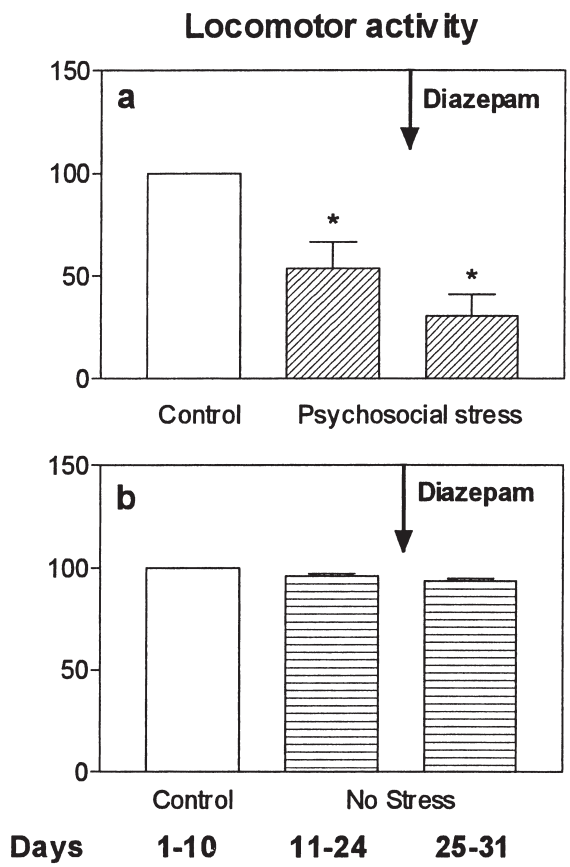

FIG. 5. Effect of psychosocial stress and diazepam treatment on locomotor activity in male tree shrews. (a) Stress + diazepam group; (b) no stress + diazepam group. For details see Fig. 1.

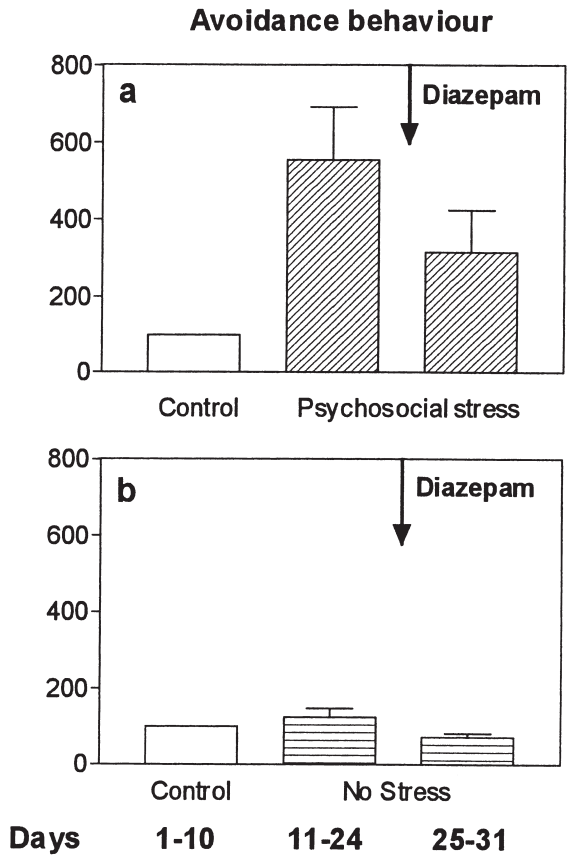

FIG. 6. Effect of psychosocial stress and diazepam treatment on avoidance behavior in male tree shrews. (a) Stress + diazepam group; (b) no stress + diazepam group. For details see Fig. 1.

larger doses markedly increase the activity resulting in elevated corticosterone levels (26,29,30,33).

Consequently, another factor that may count for the lack of therapeutical action can be the dose and, closely related, the treatment route we used. Oral administration, as used for the present investigation provides some advantages. It mimics the clinical situation that uses oral application for most patients. Due to the first path, metabolism resulted in drug and metabolite concentrations different from that obtained after IP or IV administration. The HPLC method enabled us to detect the major metabolites of diazepam, which are either hydroxylated (i.e., temazepam) or demethylated (i.e., nordiazepam); both of these products were subsequently processed to their demethylated and hydroxylated form, i.e., oxazepam. According to the metabolism of diazepam, tree shrews showed all metabolites also seen in humans or rats (18). Our results pointed towards a situation slightly different to rats where temazepam is a major metabolite (22). This gave evidence that the degradation route is more similar to humans, with the limitation that not all metabolites could be detected with our system. However, due to a fast metabolic rate, the subchronic treatment with 5 mg/kg did not give rise to long-lasting high serum levels of diazepam. On the other hand, the detection of diazepam and its metabolites 24 h after the last bolus indicated that the drug concentration had been high enough to be effective, but importantly, not sedative. In rats, analysis of serum concentrations of diazepam 35 min after IP administration (2 mg/kg) revealed titers in the range of 90 ng/ml that were not sedative during chronic administration (8). Another finding worth mentioning is the increased serum concentration of metabolites such as nordiazepam and oxazepam after 7 days of treatment in the stress + diazepam group. This finding may indicate a stress-induced change in drug metabolism that can be relevant when studying mood disorders and their pharmacological treatment.

Animal behavior studies with benzodiazepines are hampered by large variations in drug effects on behavioral variables. From various inbred mouse and rat strains that are known to differ in the sensitivity and responsiveness to benzodiazepines, it was suggested that the genetic background of the animals is responsible for the observed variations (41,45,50). Another confounding factor may arise from testing the animals outside the home cage. Transferring the animals to a novel environment, such as an open field arena or a maze, may contain stressful aspects, and only allow the detection of transient stress-induced behavioral changes (6). Finally, exposure to a brief stressful event up to at least 1 month earlier completely prevents the behavioral effects of diazepam (1). Our behavioral data of these latter findings support the fact that in situations where the individual is sensitized to stress, benzodiazepines may not be very effective because their efficacy can be markedly influenced by the ongoing stressful situation. In our control group (no stress + diazepam) we did not see any behavioral effects of diazepam. Fernandes et al. (8) reported an

effect of diazepam on locomotor activity. This effect, however, was only measurable after acute administration, and the test was done 30 min after IP injection of the drug (2 mg/kg). In our experiment, we quantified the behavior of the tree shrews about 11 h after diazepam administration (5 mg/kg PO) and 9 h after stress exposure. Based on a recent study (27), this time point was selected because it revealed the most distinct stress-induced behavioral alterations. The lack of behavioral effects of diazepam in stress-free controls may, therefore, be explained by the time elapsed between the administration of the drug and recording of the behavior.

The important finding of the present study is that a subchronic treatment of stressed tree shrews with diazepam — in a dose that did not have sedative effects in control animals, and that has been shown to be pharmacologically effective in rats (43) — did not significantly affect the stress-related endocrine and behavioral alterations. While it is simplest to attribute this result to factors such as species differences, different stress, and testing paradigms, it is also possible — but in no way proven — that in tree shrews the endogenous anxiolytic system and the endogenous stress system are functionally related.

Within the central nervous system benzodiazepines work through the GABA/benzodiazepine receptor complex to increase the inhibitory activity of GABA (51) and the agents are reported to have instantaneous anxiolytic effects (3).

Therapeutic efficacy has been shown by treatment with the tricyclic antidepressant clomipramine, which is known to inhibit 5 HT uptake. Clomipramine had a time-dependent improvement of the stress-related behavioral and endocrine changes (16). Several reports from clinical studies indicated that antidepressants can be effective in treating anxiety, and that a number of anxiolytic drugs has antidepressant actions, leading to the suggestion that there may be a common underlying mechanism between these two conditions (20,40,44). To test the hypothesis of a functional relationship between the endogenous anxiolytic system and the endogenous stress system via serotonergic mechanisms the effect psychotropic drugs such as buspirone should be evaluated in the tree shrew paradigm of psychosocial stress. Given these possibilities, further research is required to validate this nonrodent model as a tool in preclinical research of psychopathologies.

ACKNOWLEDGEMENTS

This work was supported by the DFG (Fu 174/9-1 and Hi 393/3-1).

REFERENCES

1. Antelman S. M.; Knopf, S.; Kocan, D.; Edwards, D. J.; Ritchie, J. C.; Nemeroff, C. B.: One stressful event blocks multiple actions of diazepam for up to at least a month. Brain Res. 445:380–385; 1987.
2. Aue, D.: Konfrontationen zwischen männlichen Spitzhörnchen (*Tupaia belangeri*): Konsequenzen der Sozialkontakte für Verhalten und Physiologie sowie der Einfluß individueller und äußerer Faktoren auf die Dominanzentscheidung. Thesis: Göttingen; 1988.
3. Brett, R. R.; Pratt, J. A.: Chronic handling modifies the anxiolytic effect of diazepam in the elevated plus-maze. Eur. J. Pharmacol. 178:135–138; 1990.
4. De Boer, S. F.; Van der Gugten, J.; Slangen, J. L.: Brain benzodiazepine receptor-mediated effects on plasma catecholamine and corticosterone concentrations in rats. Brain Res. Bull. 24:843–847; 1990.
5. De Boer, S. F.; Van der Gugten, J.; Slangen, J. L.: Effects of chlordiazepoxide, flumazenil and DMCM on plasma catecholamine and corticosterone concentrations in rats. Pharmacol. Biochem. Behav. 38:13–19; 1991.
6. Desan, P. H.; Silbert, L. H.; Maier, S. F.: Long-term effects of inescapable stress on daily running activity and antagonism by desimipramine. Pharmacol. Biochem. Behav. 30:21–29; 1988.
7. Diagnostic and statistical manual of mental disorders, 4th ed. Washington, DC: American Psychiatric Association; 1993.
8. Fernandes, C.; File, S. E.; Berry, D.: Evidence against oppositional and pharmacokinetic mechanisms of tolerance to diazepam's sedative effects. Brain Res. 734:236–242; 1996.
9. File, S. E.: The rat corticosterone response: Habituation and modification by chlordiazepoxide. Physiol. Behav. 29:91–95; 1982.
10. File, S. E.; Pellow, S.: The effects of putative anxiolytic compounds (PK 8165, PK 9084 and Tracozolate) on the rat corticosterone response. Physiol. Behav. 35:583–586; 1985.
11. Fischer, H. D.; Heinzeller, T.; Raab, A.: Gonadal responses to psychosocial stress in male tree shrews (*Tupaia belangeri*). Morphometry of testis, epididymis and prostate. Andrologia 17:262–275; 1985.
12. Fuchs, E.: Tree shrews. In: Poole, T., ed. UFAW handbook on the care and management of laboratory animals. Oxford, UK: Blackwell; 1999: 235–245.
13. Fuchs, E.; Flügge, G.: Modulation of binding sites for corticotropin-releasing hormone by chronic psychosocial stress. Psychoneuroendocrinology 20:33–51; 1994.
14. Fuchs, E.; Jöhren, O.; Flügge, G.: Psychosocial conflict in the tree shrew: Effects of sympathoadrenal activity and blood pressure. Psychoneuroendocrinology 18:557–565; 1993.
15. Fuchs, E.; Kleinknecht, S.: The influence of chronic social confrontation in oxygen consumption of *Tupaia belangeri* under resting conditions. Z. Säugetierkunde 51:55–57; 1986.
16. Fuchs, E.; Kramer, M.; Hermes, B.; Netter, P.; Hiemke, C.: Psychosocial stress in tree shrews: Clomipramine counteracts behavioral and endocrine changes. Pharmacol. Biochem. Behav. 54: 219–228; 1996.
17. Fuchs, E.; Schumacher, M.: Psychosocial stress affects pineal function in the tree shrew (*Tupaia belangeri*). Physiol. Behav. 47:713–717; 1990.
18. Greenblatt, D. J.; Shader, R. I.: Pharmacokinetics of antianxiety agents. In: Melzer, H., ed. Psychopharmacology. The third generation of progress. New York: Raven Press; 1987:1377–1387.
19. Henry, J. P.; Stephens, P. M.: Stress, health, and the social environment. Berlin: Springer Verlag; 1977.
20. Hoehn-Saric, R.; McLeod, D. R.: Depression and anxiety: Is there a common etiology: In: Den Boer, J. A.; Sitsen, A., eds. Handbook of depression and anxiety. New York: Marcel Dekker; 1994:119–168.
21. Hubbard, J. R.; Workman, E. A.: Handbook of stress medicine. An organ system approach. Boca Raton, FL: CRC Press; 1998.
22. Jing, X.; Wala, E. P.; Sloan, J. W.: Flunitrazepam and nordiazepam slowly released from silastic capsules induce physical dependence in rat. Drug Alcohol Dependence 39:63–67; 1995.
23. Jöhren, O.; Topp, H.; Sander, G.; Schöch, G.; Fuchs, E.: Social stress in tree shrews increases the whole-body RNA degeneration rates. Naturwissenschaften 78:36–38; 1991.
24. Jöhren, O.; Flügge, G.; Fuchs, E.: Hippocampal glucocorticoid receptor expression in the tree shrew: Regulation by psychosocial conflict. Cell. Mol. Neurobiol. 14:281–296; 1994.
25. Kawamichi, T.; Kawamichi, M.: Spatial organization and territory of tree shrews (*Tupaia glis*). Anim. Behav. 27:381–393; 1979.
26. Keim, K. L.; Sigg, E. B.: Plasma corticosterone and brain catecholamines in stress: Effect of psychotrophic drugs. Pharmacol Biochem. Behav. 6:79–85; 1977.
27. Kramer, M.; Hiemke, C.; Fuchs, E.: Chronic psychosocial stress and

antidepressant treatment in tree shrews: Time-dependent behavioral and endocrine effects. Neurosci. Biobehav. Rev. (in press).
28. Krulik, R.; Cerny, M.: Effect of chlordiazepoxide on stress in rats. Life Sci. 10:145–151; 1971.
29. Lahti, R. A.; Barsuhn, C.: The effects of minor tranquilizers on stress-induced increases in rat plasma corticosteroids. Psychopharmacologia 35:215–220; 1974.
30. Lahti, R. A.; Barsuhn, C.: The effect of various doses of minor tranquilizers on plasma corticosteroids in stressed rats. Res. Commun. Chem. Pathol. Pharmacol. 11:595–603; 1975.
31. 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.
32. Lemoine, P.; Boulenger, J. P.; Caillard, V.; Tanne, N.; Bonnet, D.: Compared efficacy of prazepam and clomipramine in major depression with anxiety: A multicenter controlled study. Pharmacopsychiatry 24:175–179; 1991.
33. Marc, V.; Morselli, P. L.: Effect of diazepam on plasma corticosterone levels in the rat. J. Pharm. Pharmacol. 21:784–786; 1969.
34. Martin, R. D.: Reproduction and ontogeny in tree shrews (*Tupaia belangeri*) with reference to their general behavior and taxonomic relationships. Z. Tierphysiol. 4:409–529; 1968.
35. Martin, R. D.: Primate origins and evolution. London: Chapman and Hall; 1990.
36. 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. Psychopharmacol. 91:467–472; 1987.
37. Molewijk, H. E.; van der Poel, A. M.; Mos, J.; van der Heyden, J. A.; Olivier, B.: Conditioned ultrasonic distress vocalizations in adult male rats as behavioral paradigm for screening anti-panic drugs. Psychopharmacology (Berlin) 117:32–40; 1995.
38. Pericic, D.; Lakic, N.; Manev, H.: Effect of diazepam on plasma corticosterone levels. Psychopharmacology (Berlin) 83:79–81; 1984.
39. Raab, A.; Storz, H.: A long term study on the impact of sociopsychic stress in tree shrews (*Tupaia belangeri)* on central and peripheral tyrosine hydroxylase activity. J. Comp. Physiol. 108: 115–131; 1976.
40. Rickels, K.; Downing, R.; Schweizer, E.; Hassmann, H.: Antidepressants for the treatment of generalized anxiety disorder. Arch. Gen. Psychiatry 50:884–895; 1993.
41. 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.
42. Rodgers, R. J.: Animal models of anxiety — Where next? Behav. Pharmacol. 8:477–496; 1997.
43. Schmitt, U.; Hiemke, C.: Combination of the open field and elevated plus-maze: A suitable test battery to assess strain as well as treatment differences in rat behavior. Prog. Neuropsychopharmacol. Biol. Psychiatry 22:1197–1215; 1998.
44. Sheehan, D. V.: The tricyclic antidepressants in the treatment of anxiety and panic disorders. Psychosomatics 27:10–16; 1986.
45. 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.
46. Sprankel, H.; Richarz, K.: Nicht-reproduktives Verhalten von *Tupaia glis* (Diard 1820) im Raum-zeitlichen Bezug. Z. Säugetierkunde 41:77–101; 1976.
47. Stöhr, W.: Heart rate of tree shrews and its persistent modification by social contact. In: Schmidt, T. H.; Dembrowski, T. M.; Blümchen, G., eds. Biological factors in cardiovascular disease. Berlin: Springer; 1986:492–499.
48. Stout, S. C.; Nemeroff, C. B.: Stress and psychiatric disorders Semin. Neurosci. 6:271–280; 1994.
49. Suetsugi, M.; Mizuki, Y.; Ushijiama, I.; Yamada, M.; Imaizumi, J.: Anxiolytic effects of low-dose clomipramine in highly anxious healthy volunteers assessed by frontal midline theta activity. Prog. Neuropsychopharmacol. Biol. Psychiatry 22:97–112; 1998.
50. Tamborska, E.; Insel, T.; Marangos, P. J.: "Peripheral" and "central" type benzodiazepine receptors in Maudsley rats. Eur. J. Pharmacol. 126:281–287; 1986.
51. Toffano, G.: Endogenous modulators of the GABA receptor. In: Enna, S. J., ed. The GABA receptors. Clifton, NJ: Humana Press; 1983:129–149.
52. Torrellas, A.; Guatra, C.; Borrell, J.: Effects of acute and prolonged administration of chlordiazepoxide upon the pituitary–adrenal activity and brain catecholamines in sound stressed and unstressed rats. Neuroscience 5:2289–2295; 1980.
53. von Holst, D.: Physiologie sozialer Interaktionen — Sozialkontakte und ihre Auswirkungen auf Verhalten sowie Fertilität und Vitalität von Tupajas. Physiol. Aktuell. 3:189–208; 1987.
54. von Holst, D.; Fuchs, E.; Stöhr, W.: Physiological changes in male *Tupaia belangeri* under different types of social stress. In: Dembroski, T. M.; Schmidt, T. H.; Blümchen, G., eds. Biobehavioral bases of coronary heart disease. Basel: S. Karger; 1983;382–390.
55. Weiner, H.: Perturbing the organism. The biology of stressful experience. Chicago: University of Chicago Press; 1992.
56. Willner, P.: Behavioral models in psychopharmacology: Theoretical, industrial and clinical perspectives. Cambridge: Cambridge University Press; 1991.
57. Willner, P.; Muscat, R.; Papp, M.: Chronic mild stress-induced anhedonia: A realistic animal model of depression. Neurosci. Biobehav. Rev. 16: 525–534; 1992.