

Effects of Buspirone and Chlordiazepoxide on Plasma Catecholamine and Corticosterone Levels in Stressed and Nonstressed Rats

SIETSE F. DE BOER, JEF L. SLANGEN AND JAN VAN DER GUGTEN

*Department of Psychopharmacology, Faculty of Pharmacy, University of Utrecht
Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands*

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DE BOER, S F, J L SLANGEN AND J. VAN DER GUGTEN. *Effects of buspirone and chlordiazepoxide on plasma catecholamine and corticosterone levels in stressed and nonstressed rats* PHARMACOL BIOCHEM BEHAV 38(2) 299-308, 1991 — The effects of intragastric administration of the prototypical benzodiazepine (BDZ) anxiolytic drug chlordiazepoxide (CDP) and the non-BDZ anxiolytic agent buspirone (BUSP) on basal and stress-elevated plasma noradrenaline (NA), adrenaline (A) and corticosterone (CS) contents were investigated. Acute dosing of CDP (1-27 mg/kg) produced dose-related increases in basal CS secretion but was without effect on basal NA levels. The high dose of CDP caused a slight short-term A increase. Dose-dependent increases in plasma A, NA and CS contents were observed after acute treatment with BUSP (2 and 20 mg/kg). A medium dose of CDP (9 mg/kg) attenuated the stress-induced CS and A elevations. High doses of CDP that elevated basal CS release prevented a further increase of CS by stress and inhibited the NA and A response to stress. BUSP (2 and 20 mg/kg) was not effective in decreasing the stress-elicited rise of CS, NA or A. Conversely, the 20 mg/kg dose of BUSP enhanced the stress-induced A response. Repeated administration of CDP (9 mg/kg/day for six days) produced tolerance to the elevation of basal CS triggered by acute CDP treatment, but increased the efficacy of the drug's CS and A attenuating action in stressed rats. Repeated administration of BUSP (2 mg/kg/day for six days) also produced tolerance to the acute BUSP-induced effect on basal CS release, but did not affect the stress-induced CS, NA and A responses. It is concluded that the clinically effective anxiolytic BUSP does not have the BDZ-like property to inhibit stress-induced elevations in CS, NA and A. Furthermore, the present data support other evidence that activation of 5-HT_{1A} receptor mechanisms increases plasma catecholamine and corticosterone concentrations.

Noradrenaline Adrenaline Corticosterone Chlordiazepoxide Buspirone Stress Rat

THE novel anxiolytic drug buspirone (BUSP) has a chemical structure and a neuropharmacological mechanism of action different from that of the prototypical benzodiazepine (BDZ) chlordiazepoxide (CDP) [see (15) for review]. BUSP, a 5-HT_{1A} receptor ligand, was reported to possess potent BDZ-like activity in animal behavioral tests of anxiolytic efficacy, and to be devoid of the sedative, anticonvulsant, muscle-relaxant and addicting effects typical of BDZs (15, 18, 48).

Whereas BDZs and BUSP both have various behavioral effects indicative of anxiolytic activity, the BDZs are also known to have effects on circulating stress-related hormones such as the glucocorticoid corticosterone (CS) and the catecholamines noradrenaline (NA) and adrenaline (A). In general, acute administration of low to moderate doses of BDZs block or attenuate stress-induced elevations in these hormones (4, 10, 16, 17, 24, 27, 28, 30, 32, 35, 39, 44, 47), while relatively high doses of BDZs cause an enhancement of CS secretion in unstressed animals (30,

32, 39, 44). The former effect has been considered to reflect the anxiolytic property of BDZs, whilst the latter effect has been attributed to their sedative/ataxic action (20, 28, 30). In accordance with this view are results regarding the effects of repeated (sub-chronic) BDZ administration on glucocorticoid secretion. Most investigators have reported that tolerance develops rapidly to the BDZ-induced elevation in basal CS concentrations but not or at least less quickly/complete to their glucocorticoid-attenuating action in stressed rats (4, 16, 28, 32).

The neuroendocrine effects of BUSP, however, have not been clearly established. Although acute administration of BUSP consistently produced dose-dependent increases in basal CS secretion (26, 33, 45), the effects of this drug on stress-induced CS elevations are scarce and conflicting; i.e., either a small inhibition (45) or no effect at all (33). In addition, no data are available concerning the effects of BUSP on basal and stress-elevated plasma NA and A concentrations. Furthermore, effects of chronic BUSP

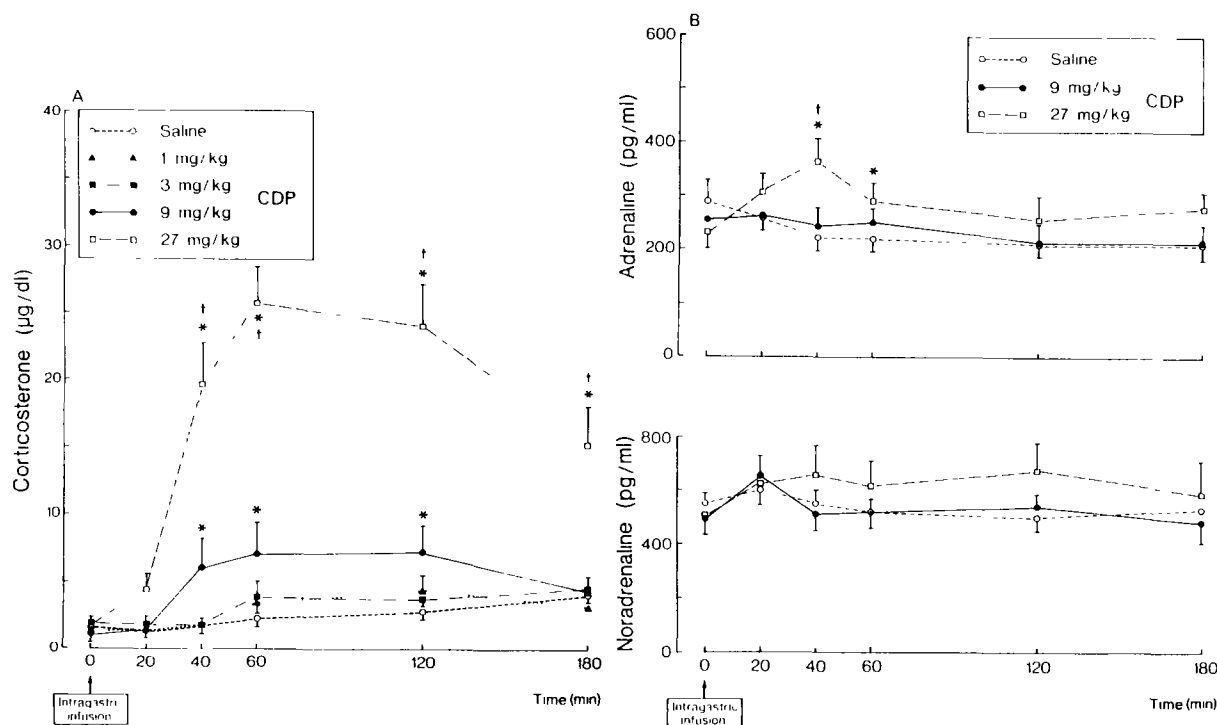


FIG 1 Time course of plasma corticosterone (A), adrenaline and noradrenaline (B) levels after intragastric administration of saline (2 ml/kg) or chlordiazepoxide (CDP, 1–27 mg/kg) to unstressed rats. Data represent mean \pm SEM ($n=12$). For the adrenaline and corticosterone data, ANOVA revealed significant Dose [A $F(2,22)=4.44$, CS $F(2,22)=41.1$] and Time [A $F(5,55)=5.26$, CS $F(5,55)=25.2$] main effects, as well as a Dose \times Time interaction [A $F(10,110)=4.24$, CS $F(10,110)=13.7$]. No significant effects were found for noradrenaline. *Significantly (at least $p<0.05$) different from saline-treated controls at similar time points or 0 time point. †Significantly different from corresponding lower drug dose values.

treatment on these stress-related hormones have not been investigated.

The present series of experiments were designed to determine the effects of acute as well as of subchronic BUSP and CDP administration on the dynamics of plasma NA, A and CS concentrations in unstressed and stressed rats.

METHOD

Animals and Housing

Male Wistar rats (Harlan-CPB, Zeist, The Netherlands) weighing 250–275 g on their arrival in the laboratory were used as subjects. They were housed individually in clear Plexiglas cages (25 \times 25 \times 30 cm) on a layer of woodshavings. For at least two weeks prior to surgery, cages were placed in a room under conditions of constant temperature ($21 \pm 2^\circ\text{C}$) and a fixed 12-h light/dark photoperiod (lights on at 7:00 a.m.). Subjects were in full view, sound and smell of each other, had free access to food and water at all times and were handled daily for weighing purposes.

Surgery and Blood Sampling

Under Hypnorm anesthesia (10 mg/kg fluanisone and 0.2 mg/kg fentanyl) and premedicated with atropine (1 mg/kg) and diazepam (5 mg/kg), animals were provided with a silastic cannula (i.d. 0.5 mm, o.d. 0.9 mm, Dow Corning, USA) into the entrance of the right atrium via an external jugular venotomy according to the techniques basically described by Steffens (40).

This cannula allows frequent withdrawal of small amounts of blood without disturbing the animal either behaviorally or physiologically (40,49). Animals were also provided with a silicon cannula (i.d. 0.8 mm; o.d. 1.4 mm) into the antrum wall of the stomach. The outer ends of both cannulae were extended subcutaneously to emerge at the top of the head and anchored to the skull (43). This indwelling catheter allows intragastric drug administration in the freely behaving and undisturbed rat. After surgery, the rats were allowed to recover for at least one week before the start of the experiments. During this period, animals were accustomed to the blood sampling procedure.

At least ninety minutes before an experiment, the indwelling cannulae were extended with polyethylene tubes (l 0.5 m; o.d. 1.45 mm; i.d. 0.75 mm) allowing blood sampling and intragastric drug infusion. Blood samples of 0.35 ml were withdrawn for determination of NA, A and CS concentrations. Immediately after each blood sample an equal volume of heparinized (12.5 IU/ml) blood, freshly obtained from a cannulated donor rat, was transfused through the catheter. At the end of the experiment, the indwelling part of the heart cannula was filled with 0.9% (w/v) NaCl containing 500 IU heparin/ml plus 60% polyvinylpyrrolidone (Merck, Darmstadt, FRG). The indwelling part of the stomach catheter was filled with 0.9% saline.

Drugs

Chlordiazepoxide hydrochloride (Hoffmann-La Roche, Basel, Switzerland) and buspirone hydrochloride (Bristol-Myers, Evansville, IN) were dissolved in physiological saline. The vehicle

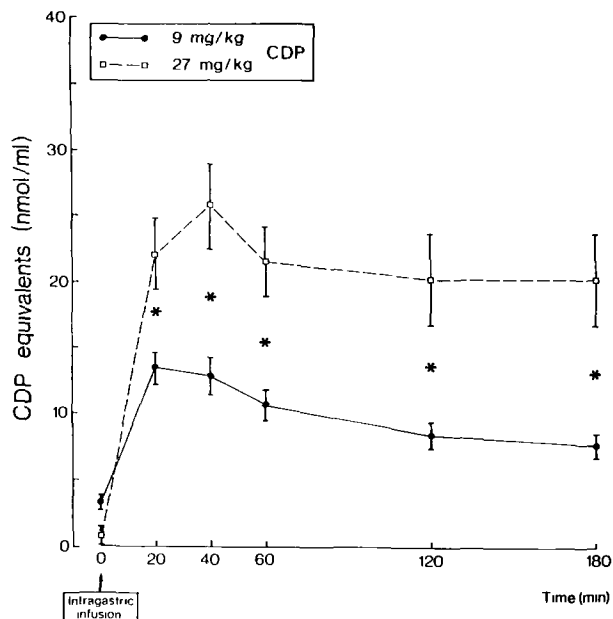


FIG 2 Time course of plasma CDP levels (as determined by benzodiazepine radioreceptor assay) following acute intragastric administration of 9 or 27 mg/kg chlordiazepoxide (CDP) to unstressed rats. Data are expressed as mean \pm SEM for 11 animals. Results from ANOVA are as follows: $F(\text{dose})(1,10) = 31.8, p < 0.001$, $F(\text{time})(5,6) = 65.4, p < 0.001$, $F(\text{dose} \times \text{time})(5,6) = 9.00, p < 0.05$. *Indicates significant (at least $p < 0.05$) differences between the two doses.

consisted of an acidified (HCl) saline solution with a similar acidity as the respective drug solutions (pH 3.2–3.9). Solutions were freshly prepared before every test. In order to minimize the non-drug-related stress associated with the most commonly used methods of drug administration (e.g., handling, IP or SC injection, orogastric intubation), drug and vehicle solutions were administered via an intragastric cannula in a volume of 2 ml/kg.

Experimental Procedures

All experiments were performed in the light period between 9:00 a.m. and 1:00 p.m.

Experiment 1 Effects of CDP and BUSP on Plasma CA and CS Levels in Unstressed Rats

Twenty subjects were used in this experiment. Twelve animals were sampled under vehicle and 4 doses of CDP (1, 3, 9 and 27 mg/kg), and eight animals under vehicle and 2 doses of BUSP (2 and 20 mg/kg), using a randomized repeated measures within-subject design with an intertest interval of 1 week. Immediately after the first blood sampling ($t = 0$ min; baseline), drug or vehicle solutions were slowly (10 s) infused via the intragastric cannula. Subsequent blood samples were taken at $t = 15, 30, 60, 120$ and 180 min following drug administration while the animals remained undisturbed in their home cages.

Experiment 2 Effects of CDP and BUSP on Novelty Stress-Induced CA and CS Elevations

Twelve subjects were used in this experiment. Six animals were sampled under vehicle and 2 doses of CDP (9 and 27 mg/

kg) and the other six under vehicle and 2 doses of BUSP (2 and 20 mg/kg), using a balanced repeated measures within-subject design with an intertest interval of 1 week. After the first blood sampling ($t = 0$), drug or vehicle solutions (2 ml/kg) were slowly (10 s) infused via the intragastric cannula. Subsequently, animals remained in their home cage for 60 min, whereafter they were picked up by hand and placed into an unfamiliar cage for 120 min (novel environment stress; NES). Blood was sampled at $t = 59, 61, 90, 120$ and 180 min after drug treatment.

Experiment 3. Effects of Acute and Repeated Administration of CDP and BUSP on Basal and Water Stress-Induced CA and CS Release

Two groups of twenty rats each were used in this experiment. Rats in each group received six consecutive daily pretreatments with either vehicle (saline, $n = 10$) or drug (9 mg/kg CDP or 2 mg/kg BUSP). During the whole experiment only one drug was used for each group. Drug and vehicle solutions were administered intragastrically at approximately the same time each day. On the test day (i.e., day 7), half of the vehicle- and drug-pretreated groups were given vehicle, whereas the other half received drug treatment. Thus for each drug-treatment group, a factorial design consisting of the following four subgroups was created (pretreatment/test treatment): A, Vehicle/Vehicle; B, Vehicle/Drug; C, Drug/Vehicle; D, Drug/Drug. On the test day, immediately after taking the first baseline blood sample (at $t = -60$ min), drug or vehicle solutions (2 ml/kg) were slowly infused via the intragastric cannula. After 59 min (at $t = -1$ min) another blood sample was taken from the unstressed animals in their home cages, whereafter (at $t = 0$ min) rats were picked up by hand and placed for 15 min into a new cage containing 5 cm of water of $35 \pm 2^\circ\text{C}$ (water stress, WS). Blood samples were taken at $t = 1$ min and $t = 15$ min. Following the $t = 15$ min blood sample, the rats were returned to their home cages and additional blood samples were taken at $t = 60$ and $t = 120$ min.

Chemical Determinations

Blood samples were immediately transferred to ice-cooled centrifuge tubes containing 10 μl heparin solution (500 IU/ml). For the determination of plasma catecholamine contents, an aliquot of 250 μl transferred blood was rapidly pipetted into chilled tubes containing 10 μl of a solution of 25 mg/ml disodium EDTA and 27.5 mg/ml reduced glutathione in order to prevent CA degradation. The remaining 100 μl blood was used for the CS and, in case of the CDP experiments, for the BDZ-receptor binding assays. After centrifugation ($4000 \times g$ for 10 min at 4°C), supernatants were removed and stored at -30°C until assayed.

The concentrations of NA and A were measured in duplicate in 20 μl perchloric acid-deproteinized plasma according to a radioenzymatic COMT-procedure (12,46). In short, the CAs were converted into their [^3H]-methoxy derivatives by incubation with S-adenosyl-L-[methyl- ^3H]methionine (80 Ci/mmol; NEN Chemicals) in the presence of catechol-O-methyltransferase. Labeled products were isolated by organic extraction and paper chromatography. After elution of the labeled products, activity was counted in a liquid scintillation analyzer (Philips, The Netherlands). CA concentrations were calculated from net DPM values for samples and internal standards and expressed as pg/ml. The intra- and interassay variabilities were less than 10% and 15%, respectively. The sensitivity of the assay was 1 pg for both NA and A.

Plasma CS concentrations were determined in duplicate according to a competitive protein-binding method (37). Corticoste-

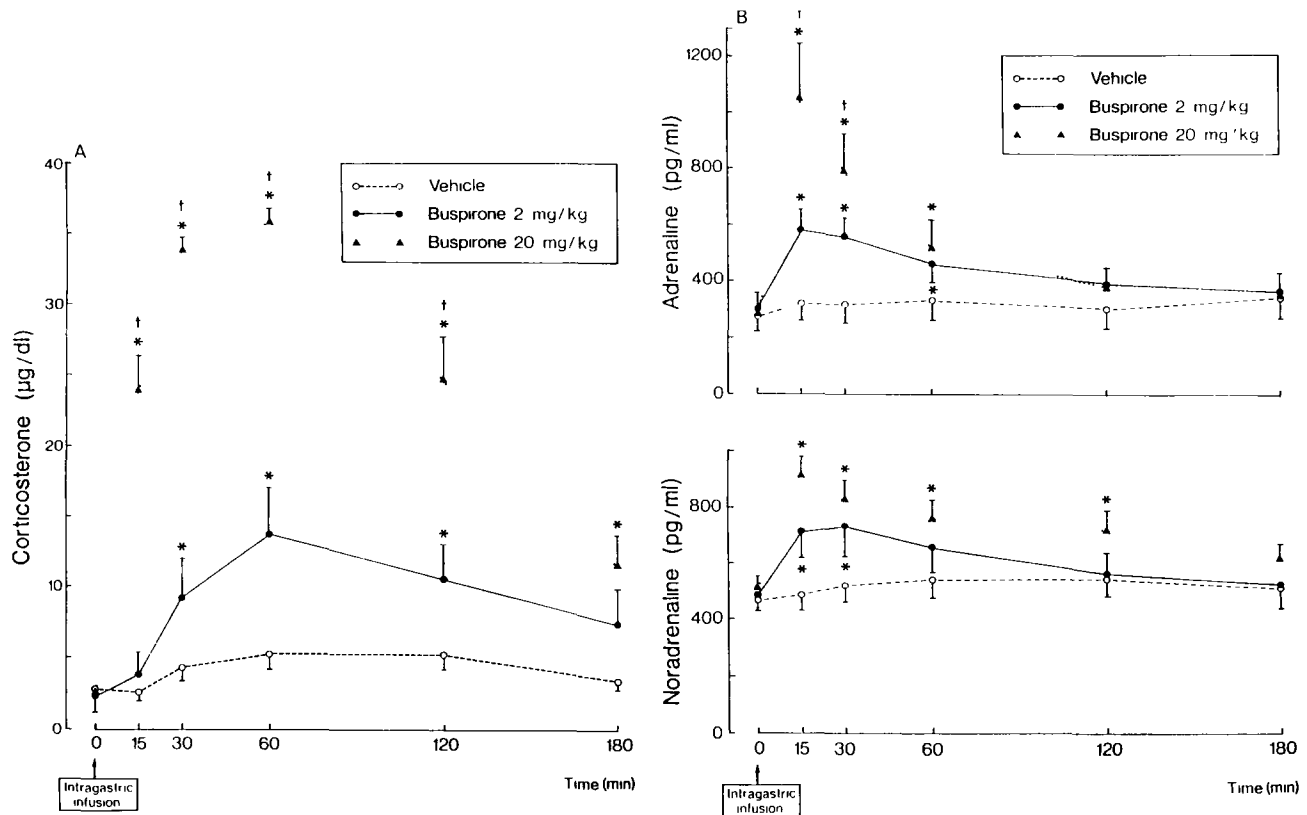


FIG 3. Time course of plasma corticosterone (A), adrenaline and noradrenaline (B) concentrations following intragastric administration of vehicle (2 mg/kg) or buspirone (BUSP, 2 and 20 mg/kg) to unstressed rats. Data represent mean \pm SEM ($n=7-8$). For all three hormones, ANOVA yielded significant main effects of Dose [NA: $F(2,12)=4.52$, A: $F(2,12)=6.89$, CS: $F(2,14)=51.9$] and Time [NA: $F(5,30)=17.7$, A: $F(5,30)=12.4$, CS: $F(5,35)=50.9$] as well as significant Dose \times Time interaction effects [NA: $F(10,60)=3.83$, A: $F(10,60)=6.67$, CS: $F(10,70)=17.8$]. *Significantly (at least $p<0.05$) different from corresponding vehicle or from time 0 value. †Significantly different from corresponding lower drug dose value.

rone was extracted with dichloromethane from 25 μ l samples of plasma and the dry residue was incubated with a corticosteroid-binding globulin tracer solution {0.1% plasma from adrenalectomized female rats containing [1,2- 3 H]-corticosterone (40–50 Ci/mmol; NEN Chemicals) as tracer}. Unbound steroid was removed using dextran-coated charcoal. Standard CS was supplied by Sigma. The intra- and interassay coefficients of variation were less than 10%. Fifty percent displacement of tracer steroid was obtained at a concentration of 20 ± 2 μ g/dl.

Benzodiazepine activity in plasma was measured according to a radioreceptor procedure (23), modified with respect to radioligand and receptor preparation (36) and adapted to small plasma samples. CDP was extracted from 50 μ l samples in 175 μ l ethyl acetate. Extract fractions were evaporated and incubated in duplicate with 900 μ l bovine frontal cortex homogenate and 25 nCi [methyl- 3 H]flunitrazepam (60 Ci/nmol; NEN Chemicals) at 4°C for 60 min. Unbound benzodiazepine was removed by rapid filtration through Whatman GF-B filters. CDP standards were used to calculate CDP equivalent levels in plasma. A plasma CDP concentration of 58 ± 6 nmol/ml resulted in 50% inhibition of specific radioligand binding.

Similarly, plasma 5-HT $_1A$ activity was measured according to a radioligand displacement method. BUSP and its metabolites were extracted into 175 μ l toluene from 25 μ l plasma to which had been added 12.5 μ l 0.5 M sodium phosphate buffer, pH 10.5. Extract fractions were evaporated and incubated in dupli-

cate with purified bovine frontal cortex homogenate and 75 nCi [propyl-2,3-ring-1,2,3- 3 H]8-hydroxy-DPAT (125 Ci/mmol, NEN Chemicals) in a final volume of 600 μ l at 37°C for 10 min. A plasma BUSP concentration of 3.1 ± 0.3 nmol/ml resulted in 50% inhibition of specific binding.

Statistical Analyses

The response-time patterns of the plasma constituents were evaluated by use of two-way analyses of variance (ANOVA) with drug treatment as within- (Experiments 1 and 2) or as between- (Experiment 3) subject factor and sampling time as repeated measures within-subject factor. The multivariate model was used for the repeated measures factor (11). Further analyses were made by paired Student *t*-tests (individual within-group comparisons) or by Duncan's new multiple range test (individual between-group comparisons) to determine the source of the detected significance in the ANOVA's (7). The criterion of significance was set at $p<0.05$.

RESULTS

Experiment 1

Under nonstress conditions, acute intragastric administration of CDP (9 and 27 mg/kg) resulted in dose- and time-related elevations of plasma CS levels (Fig. 1A; see legends to the figures

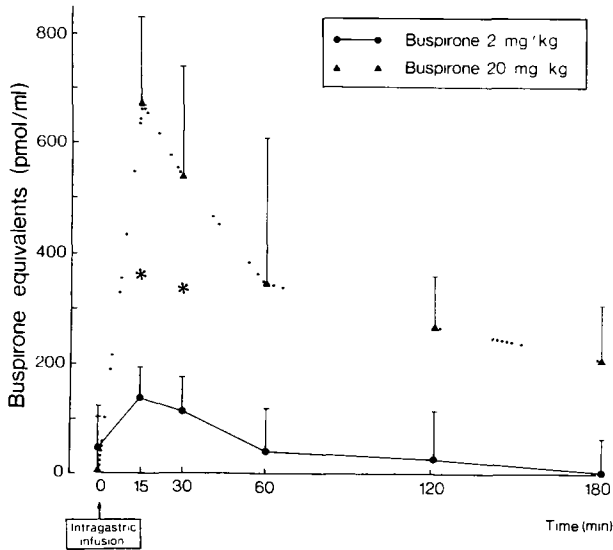


FIG 4 Time course of plasma buspirone levels (as determined by 5-HT_{1A} radioreceptor assay) following acute intragastric administration of 2 and 20 mg/kg buspirone to unstressed rats. Data are expressed as mean ± SEM for 3–8 animals. Results from ANOVA are as follows: F(dose)(1,9)=9.77, *p*<0.05, F(time)(5,5)=7.09, *p*<0.05, F(dose × time)(5,5)=2.72, *n* s. *Indicates significant (at least *p*<0.05) differences between the two doses.

for detailed results of ANOVA). These doses of CDP did not affect basal NA concentrations but a small increase in A levels was observed 40 min following the 27 mg/kg dose (Fig. 1B). Figure 2 shows the dynamics of CDP levels expressed as CDP binding equivalents in blood after administration of 9 and 27 mg/kg CDP. A clear dose-related increase was found for CDP, reaching peak levels at 20–40 min and remaining high during the rest

of the sampling period.

Marked dose- and time-dependent increases in plasma CS concentrations (Fig. 3A) as well as in plasma NA and A contents (Fig. 3B) were observed following acute administration of BUSP (2 and 20 mg/kg). Figure 4 shows the dynamics of BUSP levels expressed as buspirone binding equivalents in blood after administration of 2 and 20 mg/kg buspirone. A clear dose-related increase was found, reaching peak levels at *t* = 15 min.

Experiment 2

Exposure of vehicle-treated rats to mild stress in the form of a novel environment resulted in reliable plasma CS elevations (significant at *t* = 90 and 120 min), as well as rapid but transient increases (2.5-fold) in plasma NA (significant at *t* = 61 and 90 min) and A (significant at *t* = 61 min) concentrations (see Figs. 5 and 6). The effects of CDP and BUSP administration on these stress-elicited rises in circulating CS, NA and A concentrations are illustrated in Figs. 5 and 6, respectively. Pilot experiments showed that 1 and 3 mg/kg CDP did not affect either basal or novelty stress-elevated CS, NA and A concentrations (data not shown). As in Experiment 1, the 9 mg/kg dose of CDP caused a slight increase in basal CS levels but almost completely blocked the novelty-induced CS release. Furthermore, this dose of CDP significantly attenuated the A response to NES and tended (*p* = 0.053) to reduce the NA stress response. The high (27 mg/kg) dose of CDP strongly elevated basal CS release, but prevented a further increase by novelty stress. This dose almost completely inhibited the stress-induced increases in plasma NA and A. One hour (i.e., *t* = 61 min blood sample) after administration of 9 and 27 mg/kg CDP, plasma levels of CDP were 14.2 ± 1.6 and 29.6 ± 3.3 nmol/ml, respectively.

The 2 and 20 mg/kg doses of BUSP elevated CS values in a dose-dependent fashion. In marked contrast to CDP, BUSP was not effective in attenuating the novelty-elicited rises of plasma CS, NA and A contents. In fact, the 20 mg/kg dose of BUSP elevated basal A levels and enhanced the A response to stress (Fig. 6). One hour after administration of 2 and 20 mg/kg buspirone,

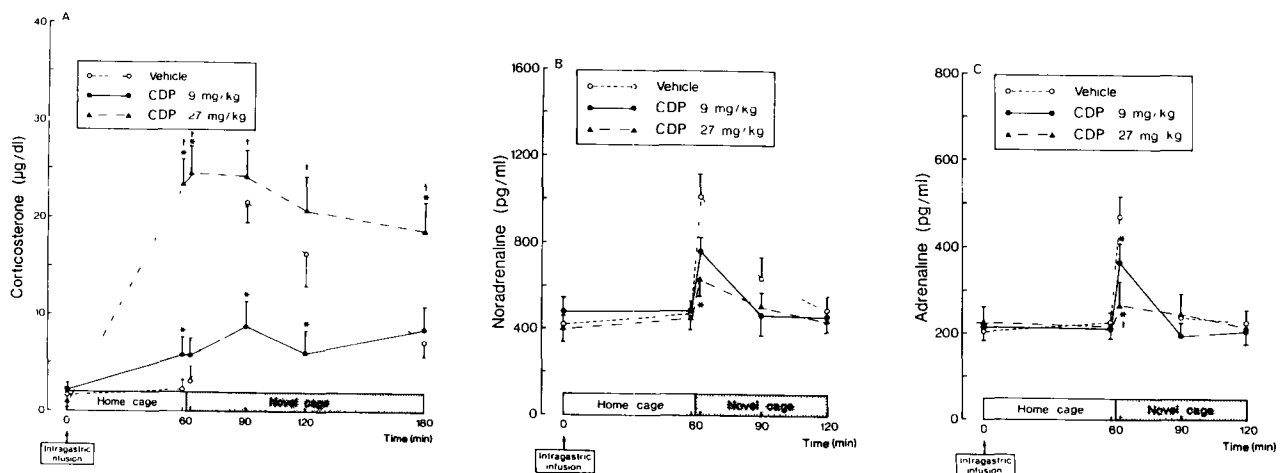


FIG 5. The effects of chlordiazepoxide (CDP, 9 and 27 mg/kg) or vehicle on plasma corticosterone (A), noradrenaline (B) and adrenaline (C) concentrations in rats under basal (home cage) condition and during exposure to a novel cage (novelty stress). Data represent means ± SEM from 6 animals. ANOVA disclosed a significant main effect of Dose for CS, *F*(2,10)=23.5, but not for NA and A. For all three hormones there was a significant main effect of Time [CS: *F*(5,25)=28.4, NA: *F*(4,20)=16.3, A: *F*(4,20)=16.1] and a significant Dose × Time interaction effect [CS: *F*(10,50)=8.67, NA: *F*(8,40)=2.51, A: *F*(8,40)=8.20]. *Significantly (at least *p*<0.05) different from corresponding vehicle value or from time 0 value. †Significantly different from the corresponding lower drug dose value.

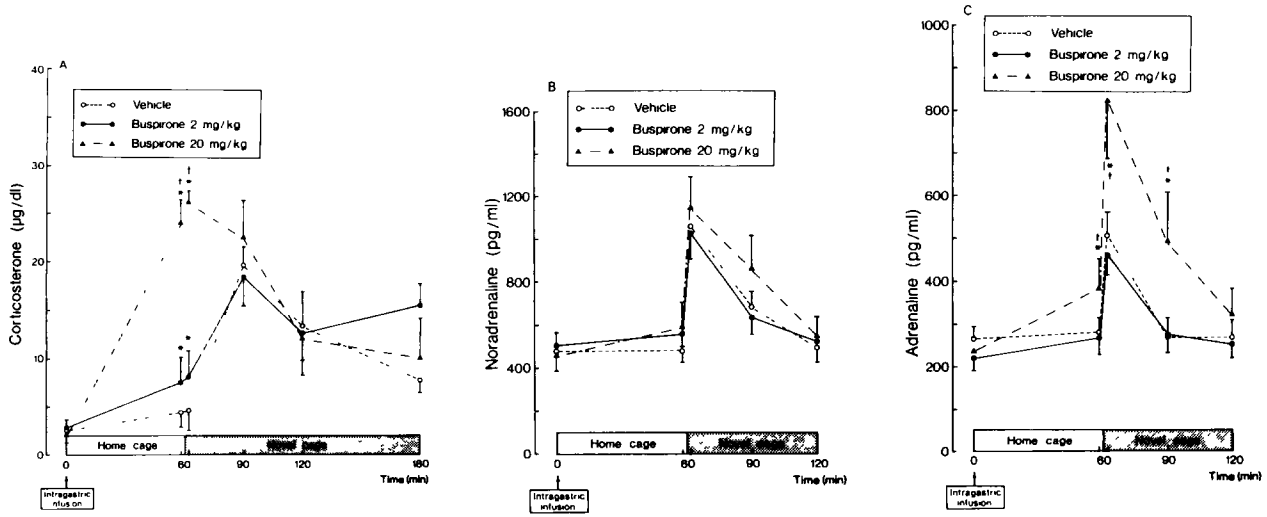


FIG 6 The effects of busprone (2 and 20 mg/kg) or vehicle on plasma corticosterone (A), noradrenaline (B) and adrenaline (C) concentrations in rats under basal (home cage) condition and during exposure to a novel cage (novelty stress). Data represent means \pm SEM from 6 animals ANOVA revealed a significant main effect of Dose for CS, $F(2,10)=9.60$, but not for NA and A. Significant main effects of Time were found for all three hormones [CS $F(5,25)=13.5$, NA, $F(4,20)=26.8$, A $F(4,20)=10.9$]. Significant Dose \times Time interaction effects were found for CS, $F(10,50)=8.10$, and for A, $F(8,40)=4.56$. *Significantly different (at least $p<0.05$) from corresponding vehicle value or from time 0 value †Significantly different from the corresponding lower drug dose value

plasma levels of BUSP were 572 ± 129 and 1278 ± 362 pmol/ml, respectively.

Experiment 3

Placement of vehicle-treated rats into shallow water for 15 min was followed by immediate and substantial increases in plasma A (4-fold) and NA (2-fold) concentrations as well as a significant rise (10-fold) in plasma CS contents (see Figs. 7 and 9). During the postwater stress period when the animals were

placed back into their home cages, a secondary increase in plasma NA occurred while plasma A and CS levels declined towards basal values. The plasma A and CS levels reached prestress values again at 60 and 120 min, respectively. The postwater stress period was characterized by vigorous grooming and wet-dog shaking behavior.

Figure 7 shows the effects of 9 mg/kg CDP administration on basal and water stress-elevated CS, NA and A release in either vehicle- or CDP (9 mg/kg/day for 6 consecutive days)-pretreated

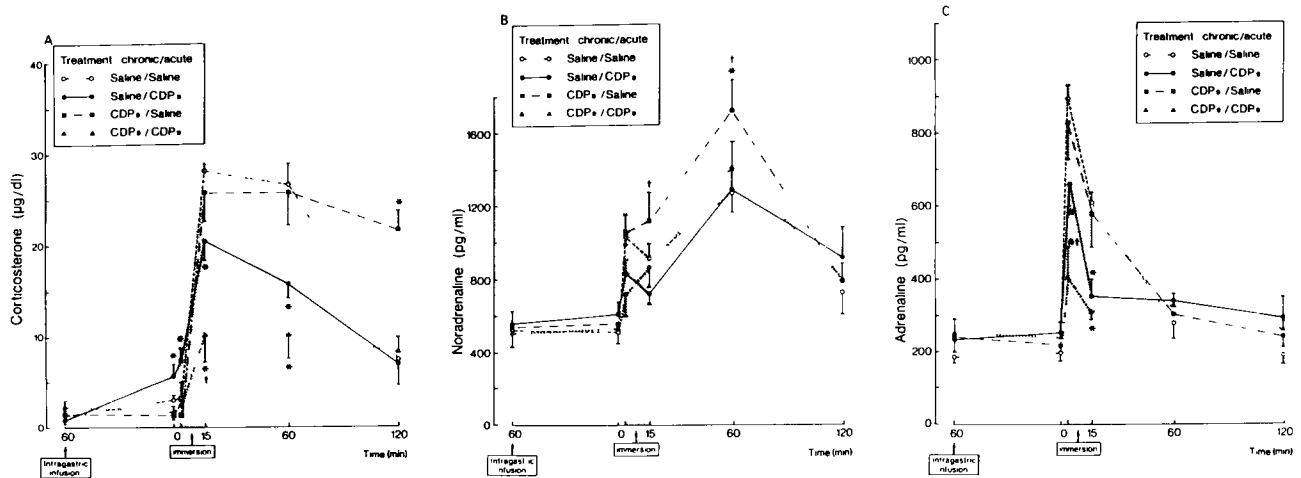


FIG 7 The effects of chlordiazepoxide (CDP, 9 mg/kg) or saline on basal and water stress-elevated corticosterone (A), noradrenaline (B) and adrenaline (C) release in both saline- and chlordiazepoxide-pretreated (9 mg/kg/day for 6 consecutive days) rats. Water-immersion period is indicated by the dotted area. Data represent means \pm SEM for 5 animals ANOVA revealed a significant main effect of Treatment for CS only, $F(3,16)=16.7$. For all three hormones there was a significant main effect of Time [CS $F(5,12)=10.9$, NA $F(5,12)=19.9$, A $F(5,12)=85.3$] and a significant Treatment \times Time interaction effect [CS $F(15,42)=6.11$, NA $F(15,42)=2.02$, A $F(15,42)=2.25$]. *Significantly (at least $p<0.05$) different from corresponding Saline/Saline control group value †Significantly different from corresponding Saline/CDP9 group value

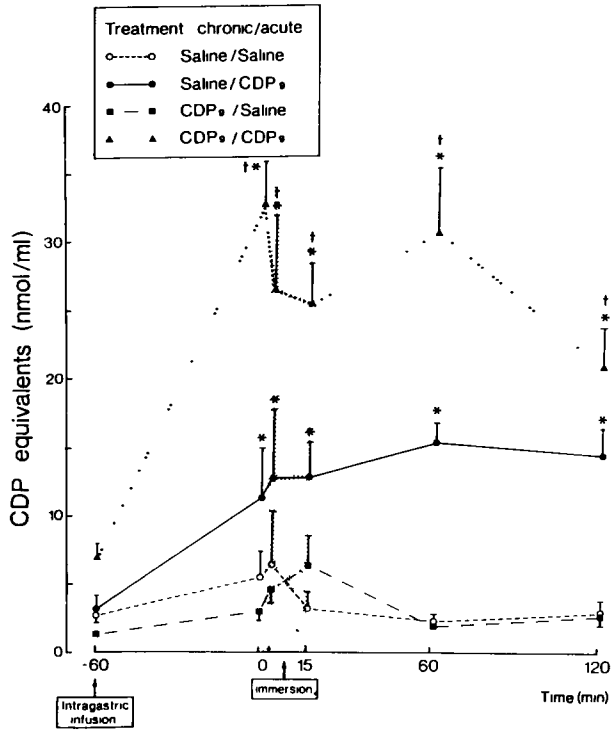


FIG 8 Time course of plasma chlordiazepoxide levels following acute administration of chlordiazepoxide (CDP, 9 mg/kg) or saline in saline- and CDP-pretreated (9 mg/kg/day for 6 consecutive days) rats during basal and water-immersion stress (dotted area) conditions. Data represent means \pm SEM for 5 animals. ANOVA yielded a significant main effect of Treatment, $F(3,15) = 62.2$, and Time, $F(5,11) = 44.8$, as well as a significant Treatment \times Time interaction effect, $F(15,39) = 6.52$. *Significantly (at least $p < 0.05$) different from corresponding Saline/Saline and CDP9/Saline group values. †Significantly different from corresponding Saline/CDP9 group value.

rats. In saline-pretreated rats the acute administration of 9 mg/kg of CDP decreased the stress-induced CS and A response. The (slight) increase in CS after acute CDP treatment was abolished in CDP-pretreated rats as compared to the vehicle-pretreated rats. In contrast, six daily CDP pretreatments significantly increased the drug's CS and A attenuating action in stressed rats. Similar to what was found in Experiment 2, neither acute nor repeated CDP (9 mg/kg) administration significantly attenuated the NA response to stress. However, vehicle administration to CDP-pretreated animals produced an enhancement of the postwater stress NA rise and a prolongation of the stress-induced CS increase. Figure 8 illustrates the dynamics of circulating CDP concentrations in the four groups of rats. In the vehicle-pretreated rats, acute CDP administration resulted in elevated plasma CDP values similar in magnitude and time pattern as in Experiment 1. Unexpectedly, significantly higher levels were found in CDP-pretreated animals following acute dosing with 9 mg/kg CDP.

The effects of 2 mg/kg BUSP or vehicle administration on basal and water stress-elevated CS, NA and A release in vehicle- and BUSP (2 mg/kg/day for 6 consecutive days)-pretreated rats are shown in Fig. 8. Six daily BUSP pretreatments produced tolerance to the acute BUSP-induced increment in basal CS secretion. As in Experiment 2, acute BUSP administration did not reduce the stress-elicited rises in CS, NA and A concentrations in either the BUSP- or Sal-pretreated group. However, irrespective of the acute treatment on day 7, chronic BUSP-pretreated rats showed an acceleration in the return towards basal levels of the stress-elevated CS concentrations. Figure 10 illustrates the dynamics of circulating BUSP concentrations in the BUSP/BUSP- and Sal/BUSP-treated rats. As in Experiment 1, acute BUSP (2 mg/kg) administration to saline-pretreated rats caused a slight elevation of BUSP levels at $t = 60$ min. In the BUSP-pretreated animals, however, acute BUSP caused no change in plasma BUSP levels.

DISCUSSION

The main new findings of this study are 1) that BUSP does not have the benzodiazepine-like property to antagonize the stress-in-

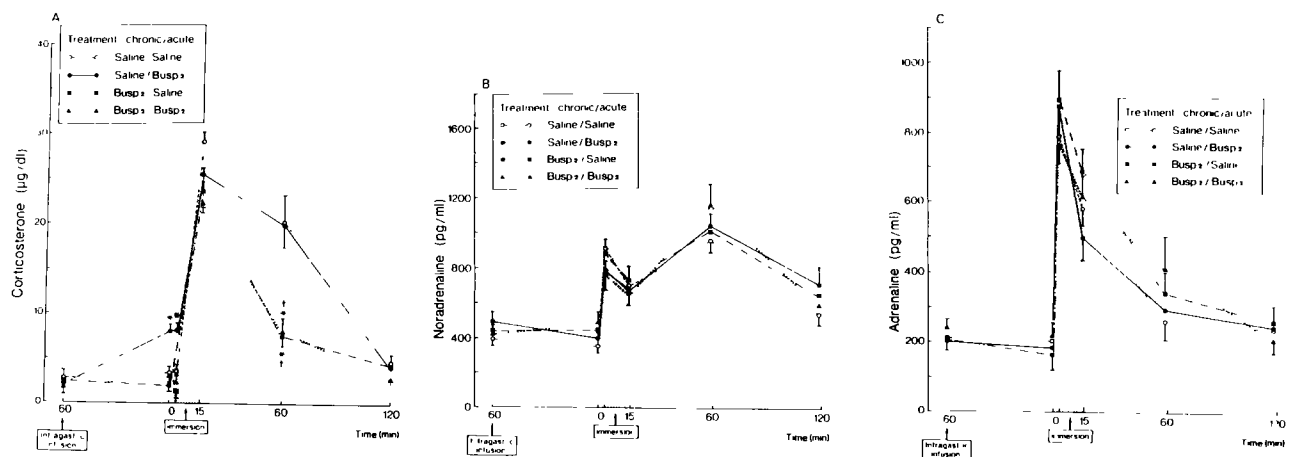


FIG 9 The effects of buspirone (2 mg/kg) or saline (2 ml/kg) on basal and water stress-elevated corticosterone (A), noradrenaline (B) and adrenaline (C) release in saline- and buspirone-pretreated (2 mg/kg/day for 6 consecutive days) rats. Data represent mean \pm SEM for 5 rats. Water-immersion stress period is indicated by the dotted area. ANOVA showed a significant main effect of Treatment for CS only, $F(3,16) = 28.6$. The main effect of Time was significant for all three hormones [CS $F(5,12) = 385$, NA $F(5,12) = 91.8$, A $F(5,12) = 124$]. The Treatment \times Time interaction was only significant for CS, $F(15,42) = 2.62$. *Significantly (at least $p < 0.05$) different from corresponding Saline/Saline group value. †Significantly different from corresponding Saline/Busp₂ group value.

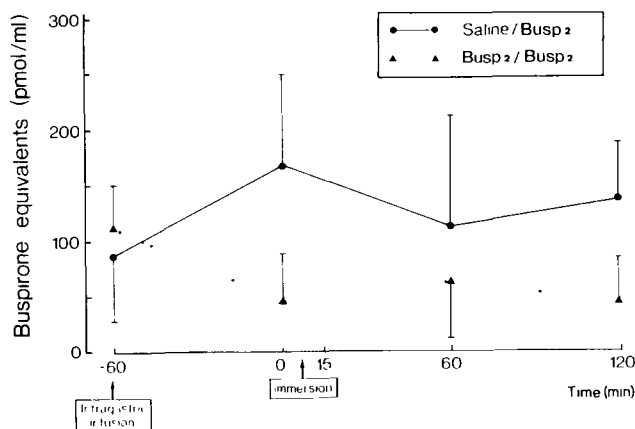


FIG 10 Time course of plasma buspirone levels following acute administration of buspirone (BUSP, 2 mg/kg) or saline in saline- and BUSP-pretreated (2 mg/kg/day for 6 consecutive days) rats during basal and water-immersion stress conditions. Data represent means \pm SEM for 5 animals. ANOVA yielded no significant main or interaction effects.

duced elevations in plasma CS, NA and A concentrations and 2) that BUSP (a 5-HT_{1A} receptor agonist), in contrast to the benzodiazepine CDP, causes marked increases in basal plasma NA and A levels. Furthermore, the results demonstrate that acute enteral administration of CDP as well as of BUSP produces dose-related increases in basal CS concentrations. These acute CS elevating effects show complete tolerance after repeated drug treatment.

The CS-releasing effects of CDP (27, 28, 32, 44) and BUSP (26, 33, 45) have previously been reported by others using different routes of drug administration (parenteral), different methods of blood sampling (decapitation) and different experimental designs (single injection test interval, between-subjects design). Although CDP and BUSP have similar effects on pituitary-adrenocortical activity in undisturbed rats, these are most likely mediated by different mechanisms of action. BUSP has been shown to produce an increase in pituitary-adrenocortical outflow by activating 5-HT_{1A} receptor mechanisms (25, 26, 38), whereas the CS-releasing action of CDP is mediated by central-type BDZ receptors (6,13) in the brain (30,32). While there is general agreement that BDZs can interact with brain 5-HT systems (4, 9, 22, 41), the importance of such an interaction as to the CDP effects on basal CS release is not known yet. Testing for the development of cross-tolerance could give an answer to this question (38).

The dose-dependent increases in plasma A and NA concentrations following BUSP administration suggest that 5-HT_{1A} receptors are involved in the regulation of neurosympathetic and adrenomedullary activity. In accordance with this suggestion are the recently reported findings that activation of the 5-HT_{1A} receptor subtype by 8-OH-DPAT (3,8) and *m*-chlorophenylpiperazine (2) similarly lead to marked increases in concentrations of NA and A in plasma. However, it is not yet clear whether the 5-HT receptors mediating such effects are located in the brain, at the sympathetic nerve endings or on the chromaffin cells of the adrenal medullae (2,8).

A number of previous studies have shown that BDZs, over a wide dose-range, attenuate the activation of the pituitary-adrenocortical axis that occurs in response to a variety of stressful stimuli, including novelty (4, 16, 17, 35), noise (28, 32, 44), restraint (24), foot-shock (5,27), and forced swimming (30,42). In contrast, information about BDZ anxiolytic drug effects on stress-induced sympatho-adrenal release of catecholamines is scarce in the

rat. One study has reported that a low (nonsedating) dose of midazolam reduced the foot-shock stress-induced plasma A but not the NA response, whereas a higher (sedative/ataxic) dose attenuated both the NA and A stress response (10). In another study it was shown that a relatively high (5 mg/kg, IP), sedative, dose of diazepam decreased the restraint stress-induced NA but not A response, whereas 1 mg/kg alprazolam inhibited both the NA and A response to restraint stress (47). The present findings that moderate to high doses of CDP oppose the novelty stress and water immersion stress-induced elevation of CS and A concentrations, whereas only high sedative doses of CDP are effective in reducing the stress-induced NA rise, are in general agreement with, and extend, the above-mentioned reports. A number of investigators have considered inhibition of stress-related hormone secretion to be predictive of or at least closely related to anxiolytic drug action at a behavioral level (24, 28, 43), while the enhanced CS concentrations in unstressed rats have been attributed to non-specific stress effects arising from sedation and ataxia (28, 35, 44). In accordance with this view are the results regarding the effects of repeated CDP treatment: tolerance develops quickly to the BDZ-induced elevation in CS concentrations but not to the drug's CS- and A-attenuating action in stressed rats [this study, (16,32)]. There is general agreement in the literature that tolerance develops very quickly to the sedative properties of BDZs, whereas its anxiolytic effects are still found after relatively short-term (i.e., one week) treatment.

Surprisingly, in the present study, it was found that repeated CDP treatment (once daily for six days) increased its potency to antagonize the stress-induced CS and A elevations. This increased potency was accompanied by a parallel increase in plasma CDP concentrations following acute dosing with CDP in these pretreated animals. Recently, a similar pharmacokinetic effect was observed in the dog, i.e., after repeated dosing with diazepam (1 or 2 mg/kg PO 3 times daily for one week), maximum drug and metabolite levels were higher than those determined after the first dosing with diazepam (31). Whether this remarkable pharmacokinetic effect after chronic BDZ pretreatment is the result of an increased rate of drug entry into the blood stream or the result of an attenuated metabolism of the drug is not clear yet. Hence, this point remains open to further investigation.

Development of tolerance is often accompanied by the occurrence of signs of withdrawal after discontinuation of drug administration (21). In the case of BDZs, spontaneous withdrawal is characterized by anxiogenic-like symptoms such as decreased food intake (29), increased body temperature (31), changes in motility (29,31), as well as increased sympathetic function (19) and raised adrenocortical activity (9,14). In the present study, CDP-withdrawal from subchronically pretreated rats did not affect basal plasma CS, NA and A concentrations, but increased the postwater-stress-induced NA response and prolonged the CS elevation in response to water stress. The data, therefore, indicate that withdrawal effects on neurosympathetic and adrenocortical outflow are only apparent under nonbasal conditions, i.e., when these systems become activated.

Although BUSP was reported to possess potent anxiolytic-like effects in several animal behavioral models as well as in man without producing the ancillary properties of the BDZs (sedation, myorelaxation, addiction), the drug was not found to be effective in attenuating the stress-elicited rise in CS and/or catecholamine concentrations in this study. Conversely, a high dose of BUSP significantly enhanced the novelty stress-induced A release. An additive effect of BUSP and stress has been observed on CS levels (33,45), but was not replicated in this study. In a previous study it was suggested that the 0.5 mg/kg (IP) dose of BUSP attenuated the CS rise in response to stress (conditioned emotional response paradigm). However, in addition to methodological prob-

lems concerning the time-lag between stressor exposure and blood sampling in that study, no significant difference was found between the CS values of the stressed vehicle-treated group and those of the stressed BUSP-treated group. Hence, the conclusion about a BUSP-mediated inhibition of stress-related CS release cannot be drawn from that study (45). Similar to our results, Matheson and co-workers (33,34) recently found that neither BUSP (1 and 10 mg/kg, IP) nor its structural analog gepirone (1 and 10 mg/kg, IP) nor its principal metabolite 1-(2-pyrimidinyl)-piperazine (1 and 10 mg/kg, IP) had the ability to blunt the response of CS to rotational stress. However, diazepam (1 and 10 mg/kg) in their hands also did not significantly alter the stress-related increase in CS, thus preventing drawing any definite conclusions from that study about whether or not a diazepam-like inhibition for BUSP of stress-related CS release exists.

Compared to BDZs, BUSP is known to have a delayed onset of action (18) and thus BUSP may need to be administered over

a period of days in order to reach full anxiolytic effectiveness. In the present study, however, a 6-day regimen of BUSP treatment did not alter the drug's inability to affect the neuroendocrine stress response, although a faster return of the stress-elevated CS levels was seen in the BUSP-pretreated rats irrespective of their acute treatment. It is concluded that the clinically effective anxiolytic BUSP does not have the BDZ-like property to inhibit stress-induced elevations in glucocorticoids and catecholamines.

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