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Plasma corticosterone responses to stress following chronic oral administration of diazepam in the rat

S. M. BARLOW*, A. F. KNIGHT AND F. M. SULLIVAN

Department of Pharmacology, Guy's Hospital Medical School, London SE1 9RT, U.K.

The effect of daily, oral administration of diazepam on plasma corticosterone responses to stressors of varying intensity was investigated. In rats exposed to the mild stress of noise, diazepam, 10 mg kg^{-1} but not 1·0 or 0·1 mg kg^{-1}, reduced plasma corticosterone concentrations by 30% in comparison with controls. However, in rats exposed to the more severe stressors, foot-shock or immobilization, none of these doses of diazepam reduced plasma corticosterone responses. In unstressed rats, diazepam 10 mg kg^{-1} raised plasma corticosterone concentrations. It is suggested that plasma corticosterone concentrations are not a reliable indicator of the tranquillizing effect of diazepam during stress.

An increase in adrenal corticosteroid secretion, as reflected by increased plasma corticosteroid concentrations, has been widely used as an index of stress in many species, including the rat. It might be expected therefore that administration of tranquillizers, such as those of the benzodiazepine group, would reduce such stress-induced increase. But in the rat the effects of the benzodiazepines on adrenocortical function are not clear. When given acutely to unstressed animals, chlordiazepoxide and diazepam in high doses cause elevation of plasma corticosteroid concentrations (Marc & Morselli 1969; Lahti & Barsuhn 1975; Keim & Sigg 1977), whilst lower doses may decrease resting plasma corticosteroid values (Krulik & Cerny 1972). After chronic treatment development of tolerance to the corticosteroid-elevating effect of high doses has been reported (Lahti & Barsuhn 1975), while continued adrenocortical stimulation has also been observed (Superstine & Sulman 1966; Marc & Morselli 1969). Stress-induced elevations in plasma corticosteroid concentrations may be reduced by acute or chronic administration of benzodiazepines (Krulik & Cerny 1971, 1972; Lahti & Barsuhn 1974, 1975; Keim & Sigg 1977) exerting a central effect preventing the release of corticotrophin. However, the reductions may be small and the effective dose-ranges are limited.

Pretreatment with benzodiazepines, in doses similar to those in the experiments just described, consistently inhibits other physiological, biochemical and pathological changes normally seen in response to stress. In rats, rabbits and monkeys, chlordiazepoxide, diazepam or nitrazepam inhibit stress-induced increases in whole brain noradrenaline turnover (Taylor & Laverty 1969; Corrodi et al 1971), and reduce hypothalamic histamine content (Taylor & Laverty 1973), hypoglycaemia and hyperlactacidaemia (Satoh & Iwamoto 1966), hypertension (Benson et al 1970), hyperthermia (Delina-Stula & Morpurgo 1970) and gastric ulceration (Haot et al 1964; Dasgupta & Mukherjee 1967; Birnbaum 1969; Schumpelick & Paschen 1974).

This raises the question of whether there may be some dissociation of the anxiolytic activity of these drugs from their effects on adrenocortical function. If this is so, then plasma corticosteroid concentrations may not be a good index of stress under such conditions.

The above studies were made using either a single dose of benzodiazepine or only one type of stress. The present experiments were therefore undertaken to investigate systematically plasma corticosteroid responses to stressors of increasing severity during chronic treatment with doses of diazepam known to inhibit many of the non-adrenocortical responses to stress. Rats were treated once daily with 0·1, 1·0 or 10·0 mg kg^{-1} per day of diazepam for 12 days during which their plasma corticosteroid responses to a mild stress (exposure to noise), a more severe stress (electric shock to the feet) and a severe stress (immobilization) were investigated.

METHODS

Thirty female rats of the Porton-Wistar strain, 175–220 g were housed, 2 animals per cage, in a ventilated room maintained at 21–23° C, under reversed lighting (white light 19·00–07·00 h, red light 07.00–19.00 h) for 1 week before and through-

* Correspondence.

out the experiment. They had free access to food and water. There was no disturbance in the animal room other than that necessitated by the treatment.

The rats were divided into 5 treatment groups, with 6 rats per group. Three groups were given 0·1, 1·0 or 10·0 mg kg^{-1} diazepam daily for 12 consecutive days, 1 group was given vehicle (1% carboxymethyl cellulose-CMC) only and 1 group was left untreated. At 10.00 h daily, each rat was weighed and the 24 h food consumption per cage was recorded. Diazepam or vehicle was then administered by gastric intubation in a volume of 0·5 ml/200 g weight. Further treatment was as follows, the first day of dosing being designated day 1.

Day 1. 1·5 h after dosing a 0·5 ml blood sample was taken from the retro-orbital sinus of each rat under brief ether anaesthesia in a room adjacent to the animal room.

Day 5. 0·5 h after dosing the rats were placed in a dark ventilated chamber and exposed to white noise of intensity 95 dB and frequency range 50 Hz–110 kHz for 1 h. At the end of this period of noise stress a 0·5 ml blood sample was taken from each rat as before.

Day 11. 1 h after dosing the rats were placed in a box with a stainless steel grid floor to which a 30 V alternating current was applied via a static scrambler circuit and exposed to mild foot-shocks of 4 s duration at a frequency of 2 shocks min^{-1} for 0·5 h. At the end of this period of foot-shock stress a 0·5 ml blood sample was taken from each rat as before.

Day 12. 0·5 h after dosing the rats were immobilized for 1 h, ventral side down, by strapping their paws onto a special board with adhesive plaster (Renaud, 1959). At the end of the period of immobilization stress a 0·5 ml blood sample was taken as before.

All blood samples were centrifuged immediately and duplicate 50 μl aliquots taken for corticosteroid assay. The remaining plasma was frozen at −25° C until assayed for diazepam using the g.l.c. method of Rutherford (1977), with the modification that benzene was used as extraction solvent and columns were packed with 2·5% OV-17. Plasma corticosterone concentrations were measured using the microfluorometric technique of Glick et al (1964).

RESULTS

Plasma corticosterone concentrations after the four test procedures on days 1, 5, 11 and 12 are shown in Fig. 1. On the first day of treatment, the mean plasma corticosterone concentration in the group given diazepam 10 mg kg^{-1} was significantly elevated compared with all other groups ($P<0·05$–0·01, Student's t-test, two-tailed).

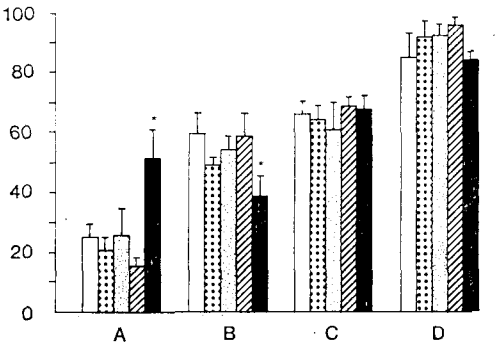

FIG. 1. Plasma corticosterone (ordinate) values (μg 100 ml) after administration of diazepam only, exposure to noise, foot-shock or immobilization stress in untreated control (open columns), vehicle control (large stippled columns), 0·1 mg kg^{-1} diazepam (small stippled columns), 1·0 mg kg^{-1} diazepam (diagonally hatched columns) and 10·0 mg kg^{-1} diazepam (closed columns) groups. Each column represents the mean value from 6 animals; vertical lines indicate s.e. mean. Significant differences between treatment groups and untreated controls are shown (*$P<0·05$, Student's t-test, two-tailed). Abscissa: (A) day 1, unstressed; (B) day 5, noise; (C); day 11, foot-shock; (D) day 12, immobilization.

On day 5, except with the diazepam 10 mg kg^{-1} group, the corticosterone values were significantly elevated in comparison with the day 1 values in all groups ($P<0·05$–0·005, paired Student's t-test). The mean corticosterone value in the diazepam 10 mg kg^{-1} group was significantly lower than that of the untreated control group on day 5. Plasma diazepam values were 33 ± 11 ng ml^{-1} (± s.e.m.) in the diazepam 10 mg kg^{-1} group. In the other two treated groups the drug was <10 ng ml^{-1}.

On day 11, the corticosterone values were elevated in comparison with corresponding day 1 values and were higher than those on day 5 in all of the treatment groups. However, there were no significant differences among treatment groups.

On day 12, significantly higher values were found than on day 11 in all 5 treatment groups ($P<0·05$–0·001, paired Student's t-test), but again there were no significant differences among treatment groups. Plasma drug concentrations were 59 ± 17, 17 ± 9 and <10 ng ml^{-1} in the 10, 1·0 and 0·1 mg kg^{-1} groups respectively.

Daily food consumption and body weight gain were similar in all groups except the diazepam 10 mg kg^{-1} group which gained weight more slowly.

DISCUSSION

The results show that chronic, daily, oral administration of diazepam in pharmacologically active doses (Zbinden & Randall 1967) failed to block increases in plasma corticosterone concentration in response to stress of varying intensity. Furthermore, no decreases in resting plasma corticosterone values were found after acute administration of diazepam to unstressed animals, whilst the highest dose used increased plasma corticosterone values, as observed by Marc & Morselli (1969) after oral dosing in the rat.

The adrenocortical stimulation produced on day 1 by the highest dose of diazepam is probably not a direct effect on the adrenal gland since pretreatment with dexamethasone has been shown to prevent this increase (Marc & Morselli 1969). It seems likely that high doses causing central depression are in themselves stressful to the animals thus causing release of corticotrophin. We observed the rats on the 10 mg kg^{-1} dose to be slightly ataxic 0·5 h after dosing. A further indication of the possible toxicity of this dose was the reduction in food intake and lower growth rate observed compared with the other treatment groups, a phenomenon seen with other psychotropic drugs at doses causing generalized central depression (Stephens 1973).

The inability of the lower doses of diazepam to decrease resting plasma corticosterone concentrations after acute oral administration on day 1 contrasts with the activity of chlordiazepoxide, which decreased plasma corticosteroids below normal resting values in the rat (Krulik & Cerny 1972) and in man (Butler et al 1968). In the present study, the plasma corticosterone concentrations of all animals were within the normal resting range for this strain of rat, sampled at the same time of day under identical conditions, of 27 ± 5 μg/100 ml ($n = 8$, unpublished observations).

There was also no evidence of diazepam inhibition of plasma corticosterone responses to stress, except in the group given 10 mg kg^{-1} exposed to the mildest stress, i.e. noise. Foot-shock was also investigated by Krulik & Cerny (1971), but at an intensity that was only mildly stressful, raising plasma corticosterone values to around 24 μg/100 ml compared with 9 μg/100 ml in unshocked controls. In their experiments, pretreatment with diazepam 5 mg kg^{-1} or chlordiazepoxide 15 mg kg^{-1} 1 h before exposure to foot-shock for 0·5 h reduced plasma corticosterone values by 20–25%.

These and the present results raise the possibility that benzodiazepines may only block plasma corticosteroid responses to mild stress, and then only partially. Lahti & Barsuhn (1974, 1975), however, using the multiple stresses of novel environment, noise and sham i.p. injection in the rat, observed plasma corticosterone concentrations of 50–90 μg/100 ml after 0·5 h of stress which could be reduced by 60–80% with diazepam 4–16 mg kg^{-1} or by 40–60% with chlordiazepoxide, 3–12 mg kg^{-1}, given i.p. 1 h before stress. Lower doses were less effective, whilst higher doses raised plasma corticosterone values above that of stressed animals not given the drug.

Inhibition of the stress response with the above doses of diazepam is at first sight rather unexpected, since Marc & Morselli (1969) have shown that diazepam, 2·5 or 5 mg kg^{-1} i.p., in the absence of other stressors, raised plasma corticosteroids. However, the timing of dosing in relation to blood sampling for corticosteroid measurement may be important. After i.p. administration, corticosteroids were high at 1 h and falling back towards control levels by 2 h (Marc & Morselli 1969). Sampling after this diazepam-induced corticosteroid peak, as in Lahti & Barsuhn's experiments, might demonstrate some inhibition of the response to other stresses. Krulik & Cerny (1971), who took serial blood samples at hourly intervals after 0·5 h of foot-shock demonstrated this probably is so in rats pretreated with chlordiazepoxide 1 h before stress when at 0–2 h post-stress the inhibition was small but by 3 h it was marked. Our failure to demonstrate inhibition of the plasma corticosteroid response to stress may be due to the later appearance of the diazepam-induced corticosteroid peak, at 2 h rather than 1 h, in the orally-dosed rat (Marc & Morselli, 1969). It is unlikely that inadequate absorption of the diazepam would explain the results since ataxia was observed within 30 min after the 10 mg dose and plasma diazepam values ranged from 15–110 ng ml^{-1} 1·5 h after the 1 and 10 mg kg^{-1} doses.

Other workers have shown that as little as one 0·4 mg kg^{-1} dose of diazepam i.p. can reduce gastric ulcer frequency by 80% in rats restrained for 22 h (Schumpelick & Paschen 1974). This dose is almost certainly below the minimum effective i.p. dose needed to inhibit plasma corticosteroid increases in response to stress (Lahti & Barsuhn 1975). Inhibition of other non-adrenocortical responses to stress, such as increases in brain noradrenaline turnover following foot-shock (Taylor & Laverty 1969) or restraint (Corrodi et al 1971; Keim & Sigg 1977) have also been obtained with 1, 5 or 10 mg

kg^{-1} diazepam s.c., doses which, as we too observed, reduced locomotor activity.

Clearly there is some dissociation between inhibition of plasma corticosteroid responses to stress and the blocking of other non-adrenocortical sequelae of stress. In view of this and the complexity of the action of benzodiazepines on adrenocortical function in the resting and stressed animal, depending upon the dose, route of administration, time of blood sampling, type of stress and possibly other unknown factors, it would seem that plasma corticosteroid concentrations are an unreliable index of the tranquillizing activity of these drugs.

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