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Benzodiazepine receptor antagonists reverse the effect of diazepam on plasma corticosterone in stressed rats

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The aim of this work has been to investigate the mechanism by which diazepam counteracts the plasma corticosterone rise induced by stress in rats. This effect is reversed by pretreatment with RO151788 and CGS8216. This observation suggests that the effect is mediated by benzodiazepine-specific receptors in brain.

The binding of benzodiazepines to their high affinity low capacity receptors in brain mediates several of their pharmacological effects (Braestrup & Squires 1977; Möhler & Okado 1977; Skolnick & Paul 1982; Mennini & Garattini 1982) as shown by the fact that antagonists of this binding (RO 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H imidazol-[1,5a][1-4-benzodiazepine-3-carboxylate]), β-carboline-3 carboxylate methylester and CGS 8216 (2-phenylpyrasolo[4,3c]-quinolin 3-(5H)one)) counteract some activities elicited by the drugs (Tenen & Hirsch 1980; Cowen et al 1981; Bernard et al 1981; Hunkeler et al 1981; Skolnick et al 1981). The anxiolytic effect attributed to the benzodiazepines seems to be concomitant with a decrease in stress-induced elevation of brain catecholamine turnover and of plasma glucocorticoids (Le Fur et al 1979; Barlow et al 1979; Krulick & Černý 1971; Marc & Morselli 1969; Lahti & Barsuhn 1974, 1975; Schallek et al 1979). Knowledge of the mechanisms underlying these effects is still scanty. RO 15-1788 is known to antagonize diazepam's action in reducing the increase in homovanillic acid induced by stress (Möhler et al 1981), but no information is available about this compound's effect on plasma glucocorticoid. We have investigated whether this effect is mediated by benzodiazepine-specific receptors in brain.

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Materials and methods

Male CD-COBS Charles River (Italy) rats, 160–180 g were kept at 22 ± 1 °C relative humidity 60% ± 5, in a light/dark cycle of 12 h. They were handled daily during the week before the experiment.

The stress was applied for 15 min and consisted in changing the animal's cage followed by making a loud noise by running a pencil over the cover. The noise was repeated 15 min later and 15 min after the rats were killed.

Diazepam was given according to two schedules: i) by oral route 30 min before stress (see Table 1); ii) by oral route the night before the experiment and 30 min before stress (see Table 2). The antagonists were given i) 45 min before stress (see Table 1) or ii) the night before and 45 min before stress (see Table 2). The compounds were dissolved in 0·5% carboxymethylcellulose. Controls (resting and stressed) received vehicle.

Plasma corticosterone was measured according to Guillemin et al (1959). Statistical significance was assessed by Duncan's test. Diazepam and RO 15-1788 were kind gifts from Roche, CGS8216 was from Ciba Geigy.

Results

The effect of diazepam on plasma corticosterone of resting rats seem to be related to the dose and the route of administration (Table 1). In our experimental conditions, diazepam given orally did not affect the concentration of plasma corticosterone, but given i.p. significantly increased it. Similar results were obtained with CGS 8216, while RO 15798 did not affect plasma corticosterone. For this reason the oral route was preferred when the effect of these compounds was challenged against stress. Table 2 summarizes the effect of diazepam, RO 15-1788 and CGS 8216 on the plasma

Table 1. Effect of diazepam, R15·1788 and CGS 8216 on rat plasma corticosterone.

Treatment† mg kg^{-1}	Plasma corticosterone μg ml^{-1} ± s.e. I.p. route controls	I.p. route treated	Oral route controls	Oral route treated
(1) Diazepam 5	5 ± 1	29 ± 8*	9 ± 1	12 ± 1
(2) Diazepam 10	10 ± 1	33 ± 5**	10 ± 1	9 ± 1
(3) Diazepam 5 × 2	—	—	8 ± 1	14 ± 3
(4) RO 15-1788 15	9 ± 1	9 ± 1	7 ± 1	9 ± 3
(5) CGS 8216 5	9 ± 1	48 ± 7**	6 ± 1	12 ± 3

Rats were killed 75 min after the last treatment

† Diazepam was given 30 min before stress and (in group 3) also the night before; RO 15-1788 and CGS 8216 were given 45 min before stress. Controls received vehicle.

* $P < 0.05$ versus controls. ** $P < 0.01$ versus controls.

Except for group 3 which consisted of 15 animals, the other groups consisted of 5 animals.

Table 2. Effect of diazepam and its antagonists on plasma corticosterone of stressed rats.

Treatment			Plasma corticosterone μg/100 ml ± s.e.				
				Stressed			
Diazepam mg kg^{-1}		Antagonist mg kg^{-1}	Resting	Controls	Diazepam	Antagonist	Diazepam + antagonist
5 × 2	RO 151788	15 × 1 oral	13 ± 1	66 ± 6†	35 ± 11‡	—	49 ± 10 n.s.
5 × 2	,,	15 × 2 oral	7 ± 1	65 ± 2†	27 ± 3‡	54 ± 6	43 ± 5**
5 × 2	,,	15 × 2 i.p.	4 ± 1	28 ± 2†	14 ± 2‡	47 ± 11	28 ± 5*
5 × 2	CGS 8216	5 × 2 oral	6 ± 1	37 ± 3†	18 ± 2‡	40 ± 4	34 ± 3••
5 × 2	,,	5 × 1 i.p.	14 ± 2	62 ± 4†	30 ± 6‡	—	47 ± 4*
5 × 2	,,	5 × 2 i.p.	7 ± 6	42 ± 5†	15 ± 3‡	57 ± 4	41 ± 6**

Diazepam was given the night before and 30 min before the stress. The antagonists were given 45 min before stress on the day of the experiment and (×2) or not (×1) the night before. Resting rats and stressed controls received only vehicle.
† $P < 0.01$ stress versus resting.
‡ $P < 0.01$ diazepam plus stress versus stress only.
** $P < 0.01$, * $P < 0.05$ diazepam plus antagonist plus stress versus diazepam plus stress.
n.s. not significant versus stress.

corticosterone of stressed rats and the interactions between antagonists and diazepam. The mild stress used induced a highly significant rise in each of the experiments reported. Pretreatment with diazepam reduced this increase. The effect was particularly evident when diazepam was given twice rather than in a single dose (data not shown).

RO 15-1788 and CGS 8216 themselves did not affect the rise of plasma corticosterone in stressed rats even at doses that increased it in resting rats.

Both RO 15-1788 and CGS 8216 reversed the effect of diazepam, CGS 8216 having an apparently more complete effect.

Discussion

As expected, the data show that diazepam reduced the increase in plasma corticosterone of stressed rats. Since diazepam itself may increase the basal plasma corticosterone concentrations of resting rats, the selection of doses and the schedule of treatment were important in making the effect significant. In those experimental conditions in which diazepam did not affect plasma corticosterone in resting rats and reduced the effect induced by stress, pretreatment with RO 15-1788 partially reversed the effect of diazepam, and CGS 8216 reversed it completely.

Since the antagonistic effect of these compounds was seen at doses that did not affect the basal level of plasma corticosterone in stressed rats, an additive type of mechanism can be ruled out. On the other hand, it has been shown that RO 15-1788 does not inhibit the plasma corticosterone rise induced by ACTH (Le Fur et al 1979). Furthermore the reversal of the effect of diazepam by RO 15-1788 and CGS 8216 might reasonably derive from their antagonizing diazepam-specific receptors in the brain (Hunkeler et al 1981; Czernik et al 1982).

These data, together with the observation that RO 15-1788 reverses the stress-induced increase in homovanillic acid (Möhler et al 1981), suggest that the anxiolytic effect of the benzodiazepines is likely to be mediated via their specific receptors in brain which are antagonized by RO 15-1788 and CGS 8216.

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