Pharmacology Biochemistry & Behavior, Vol 26, pp 561–563 Copyright © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Chlordiazepoxide and Stress Tolerance in Rats

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Received 25 June 1986

HENKE, P G *Chlordiazepoxide and stress tolerance in rats* PHARMACOL BIOCHEM BEHAV 26(3) 561–563, 1987 — Intraperitoneal injections of chlordiazepoxide in rats attenuated the severity of stomach ulcers induced by a single restraint session However, when the drug was withheld during the last session of a series of repeated restraint treatments, stomach erosions developed in the animals Vehicle-controls, on the other hand, had adapted to the chronic stress, as indexed by a decline in gastric pathology The results were discussed in reference to stress adaptation and drug withdrawal effects

Chlordiazepoxide Stress Ulcer Restraint

BENZODIAZEPINES are known to attenuate the effects of stress in a number of situations, including the pathological consequences of physical restraint on gastric functions (e.g , [2,14]). There are also some reports, however, which suggest that these anti-anxiety drugs interfere with the normal development of stress tolerance on some learning tasks (see review by Gray, Owen, Davis and Feldon [4]) The objective of the present studies was to investigate whether or not chlordiazepoxide also impairs the adaptation to repeated restraint experiences, as indexed by a decline in gastric ulceration normally seen in rats

EXPERIMENT 1

In the initial study, the aim was to test the effectiveness of various doses of chlordiazepoxide (CDP) in attenuating the gastric pathology produced by the particular restraint technique used in this laboratory.

METHOD

Forty male Wistar rats (Woodlyn Laboratories, Guelph, Ontario), 100–120 days old, were randomly assigned (n=10) to treatment conditions 5, 10, or 20 mg/kg of CDP, and vehicle control (distilled water). The animals were housed individually in a light and temperature controlled room. Lights were on from 7:00–19:00 hours.

Prior to restraint, each rat was food-deprived for 24 hours Thirty minutes after an intraperitoneal (IP) injection (2 ml/kg) with vehicle or drug, the animal was immobilized in a Plexiglas restrainer (Fisher Scientific Co , Model 01-280) for a period of four hours, starting at approximately 10·00 hours. They were individually placed in a sound-attenuating cubicle (BRS/LVE), equipped with a ventilating fan and a 15 watt light-bulb, at room temperature (approximately 21°C).

Immediately after removal from restraint, the rat was deeply anesthetized with sodium pentobarbital, and then the stomachs were removed. The stomach was opened along the greater curvature, washed in cold water, and examined microscopically under "blind" conditions. Any discontinuity in the gastric mucosa was counted as an erosion and was measured to the nearest 0.1 mm.

RESULTS AND DISCUSSION

The gastric pathology data are summarized in Table 1 It shows that, relative to the controls, the higher dosages of CDP were effective in reducing the stress-induced stomach pathology. Both 10 and 20 mg/kg CDP virtually eliminated the development of gastric erosions, whereas 5 mg/kg produced similar effects as those found in the vehicle-controls (Mann-Whitney, $p<0.05$, two-tailed tests). The ulcers were fairly shallow and did not penetrate the muscularis layer. They were only seen in the acid-secreting part of the stomach Similar results for CDP have been reported with cold-restraint (2 hours) as the stress condition [2].

The next study investigated the effects of CDP on gastric ulceration produced by repeated restraint sessions

EXPERIMENT 2

Benzodiazepines seemingly interfere with the normal development of tolerance for the effects of intermittent punishment and nonreward [4,5]. In the present study, rats received a number of restraint sessions while under the influence of CDP (10 mg/kg). The objective was to determine whether or not the drug interferes with the normal adaptation to restraint seen in rats (cf Stone and Platt [15]).

METHOD

Male Wistar rats, 100–120 days old, were randomly di-

TABLE 1
GASTRIC PATHOLOGY AFTER CHLORDIAZEPOXIDE (CDP) TREATMENT

Treatment	Percent Animals With Ulcers	Mean Number of Ulcers
5 mg/kg CDP	60	3 4
10 mg/kg CDP	10	0 3*
20 mg/kg CDP	10	0 2*
Control	50	3 2

*$p<0$ 05, Mann-Whitney, two-tailed test

TABLE 2
GASTRIC PATHOLOGY AFTER SINGLE RESTRAINT AND CHLORDIAZEPOXIDE (CDP)

Treatment	Percent Animals With Ulcers	Mean Number of Ulcers
CDP	10	0 2
Control	60	3 7*

*$p<0$ 05, Mann-Whitney, two-tailed test

TABLE 3
GASTRIC PATHOLOGY AFTER CHRONIC PRETREATMENT WITH CHLORDIAZEPOXIDE (CDP)

Treatment	Percent Animals With Ulcers	Mean Number of Ulcers
	No Restraint	
CDP	0	0
Control	0	0
	Single Restraint	
CDP	20	0 3
Control	50	3.4*

*$p<0$ 05, Mann-Whitney, two-tailed test

TABLE 4
GASTRIC PATHOLOGY AFTER REPEATED RESTRAINT AND CHLORDIAZEPOXIDE (CDP)

Treatment	Percent Animals With Ulcers	Mean Number of Ulcers
CDP	0	0
CDP—Vehicle last session	50	3 3*
Vehicle	0	0
Vehicle—CDP last session	0	0

*$p<0$ 05, Mann-Whitney, two-tailed test

vided into ten groups (n=10) Housing conditions were similar to those described in Experiment 1.

Acute CDP and Acute Stress

Two groups of rats (10 mg/kg CDP or vehicle control) received the single-restraint treatment, described in Experiment 1, to replicate the effects of the acute exposure to stress shown in the previous study

Chronic CDP Pretreatment

Two groups of rats (10 mg/kg CDP or vehicle) received a total number of eleven IP-injections Each injection was given every 48 hours, following 24 hours of food deprivation, but without restraining the animal. These animals were placed into a holding cage for 4.5 hours after the injection before being returned to their homecages Two additional groups were treated exactly the same, with the exception that after the eleventh injection, 30-min later, the rats were restrained for four hours

CDP Paired With Chronic Stress

Two groups (drug plus control) experienced eleven successive restraint sessions. Restraint (4 hr) occurred every 48 hours, following 24 hours of food deprivation, and the IP-injection of CDP (10 mg/kg) or distilled water, 30 min prior to immobilization. Immediately after the last restraint session, the stomachs were removed and inspected for pathology, as described in Experiment 1

One group received CDP for ten sessions and the vehicle-injection during the eleventh restraint period, whereas an additional group was restrained under similar conditions, but received vehicle-injections during the initial ten stress periods and the CDP-injection during the eleventh restraint session

RESULTS AND DISCUSSION

Table 2 shows the gastric pathology data for the single-restraint experience. During the single-restraint treatment, 10 mg/kg of CDP decreased the number of ulcers, replicating the results of Experiment 1 Chronic pretreatment with CDP produced similar effects under single-restraint conditions (Table 3). CDP greatly attenuated the gastric pathology, but its effect was similar to that found after an acute exposure to CDP prior to the single-restraint experience (cf. Table 2)

Table 4 summarizes the gastric pathology found after repeated restraint sessions. In the undrugged animals, repeated restraint produced an adaptation effect, shown by a decrease in ulceration. Similar stomach pathology was seen in the rats which always received the drug prior to restraint

However, as indicated in Table 4, CDP also interfered with the adaptation found in the vehicle-control animals. When CDP was withheld during the last stress session, these rats developed stomach lesions which were similar to those seen in acutely stressed control animals, i e , the drugged animals did not seem to have developed any stress tolerance during the preceding ten restraint treatments. On the other hand, an alternative explanation is that these animals experienced withdrawal reactions after CDP was withheld, which then influenced their response to stress conditions in the undrugged state

GENERAL DISCUSSION

The results of both experiments indicate that CDP can attenuate the gastric stress pathology when the drug is given prior to the restraint experience. But it also seems to interfere with the animal's adaptation to chronic stress conditions Whether or not this presumed adaptation involves learning or some form of non-associative "toughening-up" process remains to be seen [4,12], although the timing of the stress and intervening rest periods seems to be of some consequence [1] In any event, the implications for long-term therapy using CDP may be important, because this drug has also been reported to interfere with frustration tolerance under partial-reinforcement conditions, as well as the so-called partial punishment effect which under normal circumstances may also, perhaps, reflect stress tolerance [6] Furthermore, benzodiazepines have also been reported to produce amnesia-like effects in a number of testing situations [16]. Alternatively, the present results may reflect the effects of withdrawal from CDP under stressful conditions Abstinence signs, including autonomic changes, have been reported after relatively brief treatments with benzodiazepines [13].

In the past, the suggestion has sometimes been made that restraint should be classified as a physical stressor (presumably, to contrast it with "psychological" stress). But it is fairly well-established now, that this form of stress treatment affects a number of diencephalic and telencephalic brain structures, including cortical areas (e g , [3, 7, 8]) For example, multiple-unit recordings showed that forced immobilization influenced the neural activity in cortical and subcortical limbic system areas. In fact, some of these same units were also suppressed by IP-injections of CDP On the other hand, electrical stimulation of these units produced gastric ulcers in the rats [7,8]

In conclusion, the present results demonstrate that pathological changes occur in response to repeated stress when CDP is withheld during the last stress session. This effect may be due to (1) the interference with the normal adaptation to stress, or (2) the possibility that withdrawal from the drug counteracted the effects of stress adaptation It has been suggested that habituation to repeated stress is specific to that particular type of stress treatment, at least as measured by plasma prolactin and pituitary cyclic AMP [9] But other data have shown that cross-tolerance may develop, under appropriate circumstances, in the case of stress-ulcer development [10,11] Whether or not CDP also interferes with such cross-tolerance effects, however, remains to be seen.

ACKNOWLEDGEMENTS

The research was supported by grants from the Natural Sciences and Engineering Research Council of Canada

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