Pharmacology Biochemistry & Behavior, Vol. 27, pp. 35–39. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

A Benzodiazepine Receptor Inverse Agonist Inhibits Stress-Induced Ulcer Formation

RAMON TRULLAS, HILLARY GINTER AND PHIL SKOLNICK

Section on Neurobiology, Laboratory of Bioorganic Chemistry
National Institute of Diabetes, Digestive, and Kidney Diseases
National Institutes of Health, Bethesda, MD

Received 13 November 1986

TRULLAS, R., H. GINTER AND P. SKOLNICK. *A benzodiazepine receptor inverse agonist inhibits stress-induced ulcer formation.* PHARMACOL BIOCHEM BEHAV **27**(1) 35–39, 1987.—The effects of a benzodiazepine receptor inverse agonist (FG 7142) on gastric ulcer formation were studied in restrained rats. FG 7142 (10–50 mg/kg) reduced in a dose-dependent fashion both the number and cumulative length of gastric ulcers elicited by restraint for 2 hr at 4°C, but did not affect ulcer formation in unrestrained animals maintained in this environment. FG 7142 also reduced gastric ulcer formation in restrained rats maintained at 22°C for 5 hr. The ability of FG 7142 to reduce restraint-stress induced gastric ulcer formation was blocked by the benzodiazepine receptor antagonist ZK 93426 and the β-adrenoceptor antagonist propranolol. These findings suggest that FG 7142 produces a benzodiazepine-receptor mediated reduction in gastric ulcer formation, which may result from its ability to increase activity of the sympathetic nervous system.

FG 7142 ZK 93426 Restraint Stress Gastric pathology Benzodiazepine receptors Propranolol

BENZODIAZEPINE receptor "inverse agonists" (such as N-methyl-β-carboline-3-carboxamide [FG 7142] and 3-carboethoxy-β-carboline [β-CCE]) produce somatic, endocrine, and behavioral effects that are reminiscent of stress or anxiety [4, 5, 9, 17, 23]. Thus, administration of these or structurally related β-carbolines increases heart rate, blood pressure and circulating levels of stress-associated hormones. These somatic and endocrine effects are accompanied by behavioral manifestations ranging from a "proconflict" action in rodents [3] to feelings of inner tension and impending doom in normal volunteers [5]. More recent studies have demonstrated that benzodiazepine receptor inverse agonists can also produce a "learned helplessness" syndrome [6] and suppress certain aspects of immune function in rodents [1], which is reminiscent of the sequelae of inescapable shock [21]. These findings suggest that benzodiazepine receptor inverse agonists can mimic many of the effects produced by psychosocial or environmental stressors.

Forced immobilization (restraint) has been extensively used to study the neuroendocrine, neurochemical and cellular changes associated with stress [10, 11, 20, 25, 27]. In the present study, we have examined the effects of FG 7142 on gastric ulcer formation in restrained and unrestrained rats to determine whether pharmacologically-induced "stress" or "anxiety" (produced by occupation of benzodiazepine receptors in the central nervous system) would mimic or augment environmentally produced changes in gastric pathology.

We now report that FG 7142 *reduced* both the number and cumulative length of ulcers in restrained rats in a dose-dependent fashion, but did not produce or augment ulcer formation in unrestrained animals. The effect of FG 7142 in restrained rats was blocked by pretreatment with the benzodiazepine receptor antagonist ZK 93426 (5-isopropyloxy-4-methyl-3-carbomethoxy-β-carboline) [18], which suggests that this effect is mediated by occupation of benzodiazepine receptors in the central nervous system. Moreover, propranolol antagonized the protective effects of FG 7142 against ulcer formation which suggests that FG 7142 reduces restraint-stress induced ulcer formation through an increase in sympathetic tone.

METHOD

Male, Sprague Dawley rats (Charles River, Stoneridge, NY) (~200 g) were used in these experiments. Animals were housed (12/cage) in 56×42×25.4 cm metal cages in a 12 hour light cycle (lights on 0700) with food (Purina rat chow) and water freely available. Sixteen to twenty hours before restraint, the animals were transferred to another cage and food deprived, but permitted free access to water. Rats were restrained in a 4°C environment using modifications of previously described methods [10, 20, 25]. In brief, rats were restrained on 23×14 cm wooden boards fitted with metal loops to prevent head movement. Both fore- and hindlimbs were taped to metal projections which protruded 2.5 cm above the boards, and the rats transferred to a 4°C environment. In some experiments, animals were restrained at 22°C for 5 hr. FG 7142 and chlordiazepoxide were injected immediately prior to restraint, while ZK 93426, propranolol, and vehicle

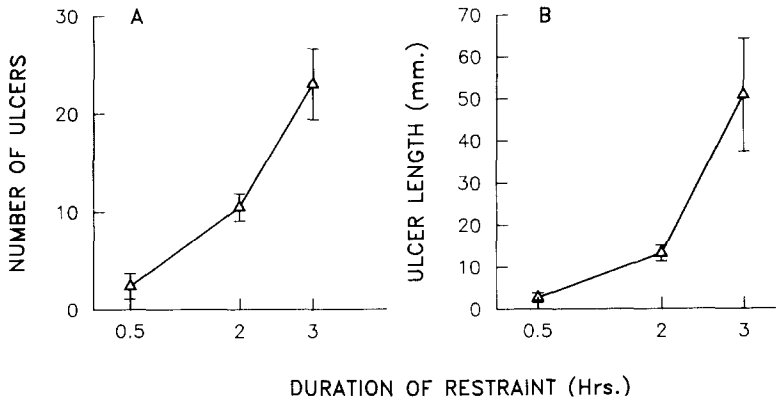

FIG. 1 Restraint-stress induced gastric ulcer formation: relationship to time of restraint. Animals were restrained at 4°C as described in the Method section for the intervals indicated. Both the number of ulcers (A) and cumulative length (B) were evaluated. Values represent mean±SEM of 6–10 rats. A statistically significant increase ($p<0.001$) in both the number and cumulative length was indicated by Kruskal-Wallis one way ANOVA.

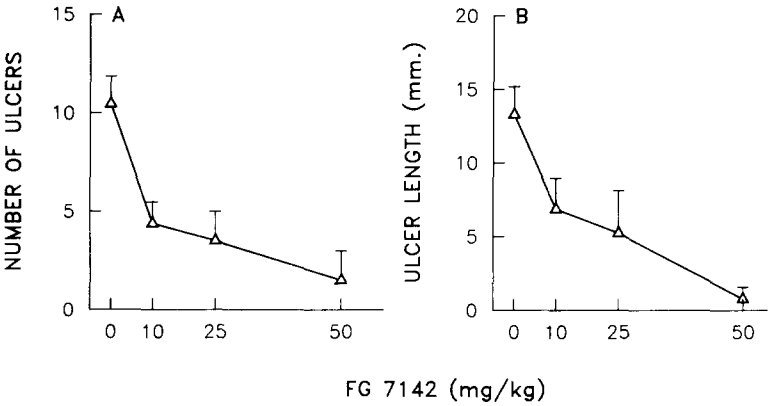

FIG. 2 Inhibition of stress-induced ulcer formation by FG 7142: Animals were administered FG 7142 (10–50 mg/kg) immediately prior to restraint. Following a 2 hr restraint (4°C), the number (A) and cumulative ulcer length (B) were evaluated as described in the Method section. Values represent mean±SEM of 10–15 animals per group. A statistically significant dose-dependent reduction in both the number ($p<0.001$) and length ($p<0.005$) of ulcers was observed (Kruskal-Wallis one way ANOVA). For comparison, a dose of 10 mg/kg chlordiazepoxide significantly reduced ulcer number to 5.11±1.5 mm ($p<0.05$, Mann-Whitney U test) in a group of 10 rats. Chlordiazepoxide reduced also the cumulative length of ulcers to 8.94±3.1 mm, although this reduction did not reach statistical significance ($p>0.1$). FG 7142 (25 mg/kg) did not produce ulcers in unrestrained rats placed in a 4°C environment for 2 hr (data not shown).

were injected 10 min prior to restraint. All drugs were administered intraperitoneally in a volume of 1 ml/kg.

Animals were sacrificed by decapitation immediately after removal from the restraining apparatus. The stomachs were removed and clamped with a hemostat just above the cardiac sphincter, and then filled with saline by inserting a needle through the pylorus. The inflated stomachs were placed in a 10% formalin bath for 45–60 seconds, and then opened along the greater curvature. The stomachs were then stretched and pinned on a styrofoam board, and the presence and length of ulcers determined with an Olympus CH microscope at a 40× magnification. The ulcers were counted and the length measured along the greater axis using a calibrated eyepiece.

Statistical analyses were performed using the Kruskal-Wallis one way Analysis of Variance (ANOVA) and Mann-Whitney U tests for comparisons between groups.

FG 7142 was purchased from Research Biochemicals Inc., Wayland, MA. Propranolol HCl was purchased from Sigma Chemical Co., St. Louis, MO. ZK 93426 was donated by Schering AG Berlin, and chlordiazepoxide HCl by Hoffmann-LaRoche, Nutley, NJ. Drugs were dissolved or suspended in 10% diluted emulphor-90% phosphate buffered saline [23].

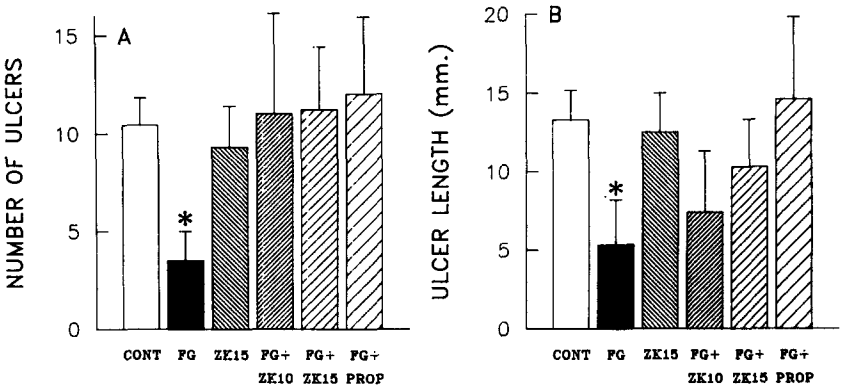

FIG. 3 Reduction in restraint-stress induced ulcers by FG 7142: reversal by ZK 93426 and propranolol. Animals were injected with either propranolol HCl (2 mg/kg) or ZK 93426 (10 mg/kg and 15 mg/kg), and ten minutes later were administered FG 7142 (25 mg/kg). The rats were then restrained for 2 hr at 4°C as described in the Method section and the number and length of ulcers evaluated. Values represent mean±SEM of 8–10 animals per group. Symbols: *=Significantly different from control group, $p<0.005$, Mann-Whitney U test.

RESULTS

Head and limb immobilization in a 4°C environment produced a time dependent increase in both the number (Chi-square=13.17 $p<0.001$) and cumulative length (Chi-square=14.8, $p<0.001$) of ulcers (Fig. 1). In contrast, no ulcers were observed in unrestrained animals maintained at this temperature (Fig. 1, legend). The effects of benzodiazepine receptor ligands on ulcer formation were studied after a two hour restraint period, since this interval produced robust increases in both the number and average length of ulcers, and readily permitted the detection of both increases and decreases in ulcer formation.

FG 7142 reduced in a dose-dependent fashion (10–50 mg/kg) both the number (Chi-square=14.9, $p<0.001$) and cumulative length (Chi-square=12.5, $p<0.005$) of ulcers induced by two hours of restraint at 4°C. The lowest dose of FG 7142 reduced the number of ulcers by ~58% ($p<0.005$) and their cumulative length by ~48% ($p<0.05$), respectively. At the highest dose employed (50 mg/kg) FG 7142 reduced the number and length of ulcers by ~85% ($p<0.01$) and ~94% ($p<0.01$), respectively (Fig. 2). By comparison, chlordiazepoxide HCl (10 mg/kg) reduced the number of ulcers formed by ~51% ($p<0.01$). The cumulative length of ulcers was also reduced (~33%) by chlordiazepoxide, but this effect did not reach statistical significance ($p<0.1$) (Fig. 3, legend).

The benzodiazepine receptor antagonist ZK 93426 [18] (10 and 15 mg/kg) blocked both the reduction in number and length of ulcers observed with FG 7142 (25 mg/kg), but did not significantly affect either ulcer number or cumulative length when administered alone to restrained rats (Fig. 3). The β-adrenoceptor antagonist propranolol (2 mg/kg) also blocked the effects of FG 7142 (25 mg/kg) at a dose which had been reported not to affect ulcer formation in restrained rats [7].

The effects of FG 7142 were also examined following a 5 hr ambient temperature restraint. This condition produced a number and cumulative length of ulcers comparable to that obtained in the shortest period (0.5 hr) of restraint in a 4°C environment (Table 1). Under these conditions, FG 7142 (50 mg/kg) completely abolished the formation of ulcers, while chlordiazepoxide (10 mg/kg) reduced the number and length of ulcers by ~67% ($p<0.05$) and ~86% ($p<0.05$), respectively (Table 1).

TABLE 1

THE EFFECTS OF BENZODIAZEPINE RECEPTOR LIGANDS ON RESTRAINT-STRESS INDUCED ULCER FORMATION

Treatment	Number of Ulcers	Length (mm)
Vehicle	2.69 ± 0.8	3.4 ± 1.1
Chlordiazepoxide (10)	0.88 ± 0.3*	0.8 ± 0.3*
FG 7142 (50)	0*	0*

Animals were injected with chlordiazepoxide HCl, FG 7142, or vehicle 10 min prior to restraint. Animals were restrained as described in the Method section for 5 hr at 22°C. The doses of drug (mg/kg) used are in parentheses. Each group consisted of 8–12 animals. *=Significantly different from vehicle group, $p<0.05$, Mann-Whitney U test.

DISCUSSION

Benzodiazepine receptor "inverse agonists" mimic some of the somatic, endocrine, behavioral and cellular changes produced by either environmental or psychosocial stressors [1, 3–6, 17, 23]. The present studies were designed to determine whether the benzodiazepine receptor inverse agonist FG 7142 could mimic the effect of restraint stress to promote the formation of gastric ulcers. In preliminary experiments, it was observed that FG 7142 (25 mg/kg) did not produce ulcers in unrestrained rats exposed to a 4°C environment for 2 hr (Fig. 2, legend). Furthermore, rather than acting synergistically with restraint to promote ulcer formation, FG 7142 *inhibited* the development of gastric ulcers in a dose-dependent fashion (Fig. 2), and in this respect resembles moderate doses of benzodiazepine receptor agonists such as chlordiazepoxide (Table 1, Fig. 3 legend, and [8, 12, 13]).

However, File and Pearce [8] and Ushijima *et al.* [28] have shown that lower doses (within the anxiolytic dose range) of chlordiazepoxide, diazepam, and clonazepam *exacerbate* stress-induced ulcer formation. The reduction in ulcers by FG 7142 was also observed in rats restrained at ambient temperature (Table 1), suggesting that this effect is not specific to a particular set of restraint conditions.

The ability of ZK 93426 to block the effects of FG 7142 on ulcer formation suggests this action of FG 7142 is initiated at benzodiazepine receptors in the central nervous system (CNS), since ZK 93426, like the benzodiazepine receptor antagonist RO15-1788 [16], binds to benzodiazepine receptors with high affinity [18,24] and blocks the pharmacologic effects of both benzodiazepines and benzodiazepine receptor inverse agonists. The lack of effect of ZK 93426 on ulcer formation when administered alone is consistent with the lack of "intrinsic" actions of this compound [2] at the doses employed in this study [18].

The observation that propranolol blocks the effects of FG 7142 on stress-induced ulcer formation (Fig. 3) at a dose which does not exacerbate ulcer formation [7] may help explain the apparently anomalous action of FG 7142 to reduce stress-induced ulcer formation. Several studies [7, 14, 19, 26] suggest that the sympathetic nervous system may be an important modulator of stress-induced ulcer formation (cf. [15] for review). For example, parenteral administration of β-adrenoceptor agonists such as isoproterenol and salbutamol antagonize stress-induced ulcer formation [7], effects that are reversed by β-adrenoceptor blockers such as propranolol [7]. Furthermore, sympathectomy induces gastric lesions and aggravates stress-induced ulcer formation [14, 15, 19]. While the effects of propranolol could be attributed to its membrane stabilizing properties, the doses required to produce this effect far exceed those necessary for β-adrenoceptor blockade [22], and would not be achieved after the dose (2 mg/kg) used in the present study. Since benzodiazepine receptor inverse agonists like FG 7142 increase sympathetic tone [4, 5, 17, 23], this benzodiazepine-receptor mediated activation of the sympathetic nervous system could be responsible for the blockade of stress-induced ulcers. Conversely, the increases in stress-induced ulcer formation observed after low doses of benzodiazepines [8,28] may be attributable to the ability of these compounds to reduce the increased sympathetic tone associated with stress or anxiety [4]. This hypothesis is currently under investigation. These observations do not rule out the possibility that FG 7142 blocks stress-induced ulcer formation through (e.g.) an effect on hormones such as glucocorticoids which can also influence ulcer formation [14], or that this effect is mediated through activation (via occupation of benzodiazepine receptors) of other C.N.S. pathways. However, the findings that parenterally administered isoproterenol attenuates, and sympathectomy exacerbates, ulcer formation [15] support the concept of a centrally (i.e., benzodiazepine receptor) mediated activation of peripheral catecholamine pathways to attenuate ulcer formation.

ACKNOWLEDGEMENTS

R.T. acknowledges the financial support of the Comite Conjunto Hispano-Norteamericano and The Burroughs Wellcome Fund. The authors wish to thank Michael Jackson and Barrington Jackson for excellent technical assistance.

REFERENCES

1. Arora, P., E. Hanna, S. Paul and P. Skolnick. Suppression of the immune response by benzodiazepine receptor inverse agonist. *J Neuroimmunol*, in press, 1986.
2. Cooper, S J. and W. R. Moores. Chlordiazepoxide-induced hyperphagia in non-food-deprived rats: effects of Ro15-1788, CGS 8216 and ZK 93426. *Eur J Pharmacol* **112:** 39–47, 1985.
3. Corda, M., W. Blaker, W. Mendelson, A. Guidotti and E. Costa. β-Carbolines enhance shock-induced suppression of drinking in the rat. *Proc Natl Acad Sci USA* **80:** 2072–2078, 1983.
4. Crawley, J., P. Ninan, D. Pickar, G. Chrousos, M. Linnoila, P. Skolnick and S. Paul. Neuropharmacological antagonism of the β-carboline-induced "anxiety" response in rhesus monkeys. *J Neurosci* **5:** 477–485, 1985.
5. Dorow, R., R. Horowski, G. Paschelke, M. Amin and C. Braestrup. Severe anxiety induced by FG 7142, a β-carboline ligand for benzodiazepine receptors. *Lancet* **i:** 98–99, 1983.
6. Drugan, R., S. Maier, P. Skolnick, S. Paul and J. Crawley. A benzodiazepine receptor antagonist induces learned helplessness. *Eur J Pharmacol* **113:** 452–457, 1985.
7. Esplugues, J., J. Lloris, E. Marti-Bonmati and E. Morcillo. Effects of β-adrenoceptor drug stimulation on various models of gastric ulcer in rats. *Br J Pharmacol* **76:** 587–594, 1982.
8. File, S. and J. Pearce. Benzodiazepines reduce gastric ulcers induced by stress. *Br J Pharmacol* **74:** 593–599, 1981.
9. File, S. and S. Pellow. The effects of putative anxiogenic compounds, FG 7142, CGS 8216, and RO 15-1788 on the rat corticosterone response. *Physiol Behav* **35:** 587–590, 1985.
10. Glavin, G. Restraint ulcer. History, current research and future implications. *Brain Res Bull* **5:** Suppl 1, 51–58, 1980.
11. Glavin, G., M. Tanaka, A. Tsuda, Y. Kohno, Y. Hoaki and N. Nagasaki. Regional rat brain noradrenaline turnover in response to restraint stress. *Pharmacol Biochem Behav* **19:** 287–290, 1983.
12. Gupta, M., R. Nath, G. Gupta and K. Bhargava. A study of the anti-ulcer activity of diazepam and other tranquillosedatives in albino rats. *Clin Exp Pharmacol Physiol* **12:** 61–66, 1985.
13. Haot, J., B. Djahanguiri and M. Richelle. Action protectrice du chlordiazepoxide sur l'ulcere de contrainte chez le rat. *Arch Int Pharmacodyn* **148:** 557–559, 1964.
14. Hernandez, D., J. Adcock, C. Nemeroff and A. Prange. The role of the adrenal gland in cytoprotection against stress-induced gastric ulcers in the rat. *J Neurosci Res* **11:** 193–201, 1984.
15. Hernandez, D. Neuroendocrine mechanisms of stress ulceration: focus on thyrotropin releasing hormone (TRH) (minireview). *Life Sci* **39:** 279–296, 1986.
16. Hunkeler, W., H. Mohler, L. Pieri, P. Polc, E. Bonetti, R. Cumin, R. Schaffner and W. Haefely. Selective antagonists of benzodiazepines. *Nature* **290:** 514–516, 1981.
17. Insel, T., P. Ninan, J. Aloi, D. Jimerson, P. Skolnick and S. Paul. A benzodiazepine receptor-mediated model of anxiety. Studies in non human primates and clinical implications. *Arch Gen Psychiatry* **41:** 741–750, 1984.
18. Jensen, L., E. Petersen, C. Braestrup, T. Honoré, W. Kehr, D. Stephens, H. Schneider, D. Seidelmann and R. Schmiechen. Evaluation of the β-carboline ZK 93426 as a benzodiazepine receptor antagonist. *Psychopharmacology (Berlin)* **83:** 249–256, 1984.
19. Kasuya, Y., T. Marata and S. Okabo. Influence of surgical sympathectomy on gastric secretion and ulceration in rats. *Jpn J Pharmacol* **28:** 297–304, 1978.
20. Kvetnasky, R. and L. Mikulaj. Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinology* **87:** 738–742, 1970.

21. Laudenslager, M., S. Ryan, R. Drugan, R. Hyson and S. Maier. Coping and immunosuppression: inescapable but not escapable shock suppresses lymphocyte proliferation. *Science* **221:** 568–570, 1983.
22. Nickerson, M. and B. Collier, Drugs inhibiting adrenergic nerves and the structures innervated by them. In: *The Pharmacological Basis of Therapeutics,* 5th edition, edited by L. Goodman, A. Gilman, A. Gilman and G. Koelle. New York: MacMillan Pub. Co., 1975, pp. 533–564.
23. Ninan, P., T. Insel, R. Cohen, J. Cook, P. Skolnick and S. Paul. Benzodiazepine receptor-mediated experimental "anxiety" in primates. *Science* **218:** 1332–1334, 1982.
24. Stephens, D. N., W. Kehr, H. H. Schneider and R. Schmiechen. β-Carbolines with agonistic and inverse agonistic properties at benzodiazepine receptors of the rat. *Neurosci Lett* **47:** 333–338, 1984.
25. Stone, E. A. and J. Platt. Brain adrenergic receptors and resistance to stress. *Brain Res* **237:** 405–414, 1982.
26. Taylor, J. and G. Nabi Mir. Alpha adrenergic receptors and gastric function. *Drug Dev Res* **2:** 105–122, 1982.
27. Usdin, E., R. Kvetnansky and J. Axelrod (editors). *Stress: The Role of Catecholamines and Other Neurotransmitters, Vols I and II.* New York: Gordon and Breach Scientific Publishers, 1984.
28. Ushijima, I., Y. Mizuki, T. Hara, R. Kudo, K. Watanabe and M. Yamada, The role of adenosinergic, GABAergic and benzodiazepine systems in hyperemotionality and ulcer formation in stressed rats. *Psychopharmacology (Berlin)* **89:** 472–476, 1986.