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BRIEF COMMUNICATION

Evidence for Intrinsic Behavioural Activity of the Benzodiazepine Antagonist, Ro15-1788, in Male Mice

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RODGERS, R. J., A. J. WATERS AND S. ROSENFIELD. *Evidence for intrinsic behavioural activity of the benzodiazepine antagonist, Ro15-1788, in male mice.* PHARMACOL BIOCHEM BEHAV 19(5) 895–898, 1983.—It would be predicted that putative benzodiazepines should be released under anxiety-provoking conditions and that behavioural changes should be observed following pretreatment with selective antagonists of the benzodiazepine receptor. To test this hypothesis, adult male albino mice were briefly exposed to a novel, brightly-illuminated arena during the dark phase of their LD cycle. Under these test conditions, Ro15-1788 (10 mg/kg) enhanced total rearing whilst, at 5–10 mg/kg, it significantly altered the normal pattern of rearing over the test session. However, at the highest dose tested (20 mg/kg), such behavioural changes were no longer apparent. A similar, though non-significant, trend was observed for locomotor activity. These data, the first to demonstrate low-dose intrinsic activity of Ro15-1788 in mice, suggest that benzodiazepine antagonists may prove to be powerful tools in the study of the behavioural significance of the benzodiazepine receptor.

Ro15-1788 Benzodiazepine antagonist Activity Exploration Mice

IT is currently believed that benzodiazepines exert their effects via an interaction with specific, high-affinity binding sites in the central nervous system [19, 25, 26]. Recently, three other groups of compounds (i.e. the imidazobenzodiazepines, β-carbolines and pyrazoloquinolines) have been found to possess high affinity for these receptors *in vitro*, but to antagonize the actions of conventional benzodiazepines *in vivo* [2, 3, 5, 6, 8, 15, 23]. Of particular relevance to the current study is the report that the imidazobenzodiazepine derivative Ro15-1788 (ethyl-8-fluoro-5-6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate) potently inhibits ^{3}H-diazepam binding in rat brain yet, in a wide variety of species, produces none of the characteristic effects of benzodiazepine drugs [3,15]. However, in neurological and behavioural studies, Ro15-1788 has been found to act as a potent and selective benzodiazepine antagonist, preventing and reversing the effects of a number of benzodiazepines [3, 5, 6, 9, 13, 14, 15, 16, 17, 22, 28, 29].

Although Ro15-1788 is widely believed to be devoid of intrinsic activity at low doses [3, 6, 16, 18, 20, 24, 28, 29], two recent behavioural studies in rats have questioned this assertion. In the social interaction test of anxiety, Ro15-1788 (10 mg/kg) induces a weak, though significant, anxiogenic effect [11] whilst, in the hole-board exploration test, it exerts a dose-dependent (4–20 mg/kg) stimulatory action [12]. These important new findings suggest that, under appropriate test conditions, and in a manner analogous to the utility of naloxone in the study of endogenous opioids, this compound may help to reveal the functional significance of putative endogenous benzodiazepines. We now report that Ro15-1788 also possesses low-dose intrinsic behavioural activity in male mice tested in a novel environment.

METHOD

Subjects

Sixty-eight adult male BKW albino mice (Swiss-derived; 30–50 g), from Bradford University Breeding colony, were used. Subjects were group-housed (10/cage) for one week prior to testing, with food and water available ad lib. They were maintained in a temperature-controlled room (24±1°C), in which a reversed light/dark cycle (12 hr) was in operation (lights on: 1900 hr). All testing took place during the mid-portion of the dark phase.

Apparatus and Procedure

A rectangular enclosure (37.5×27×26.5 cm high) served

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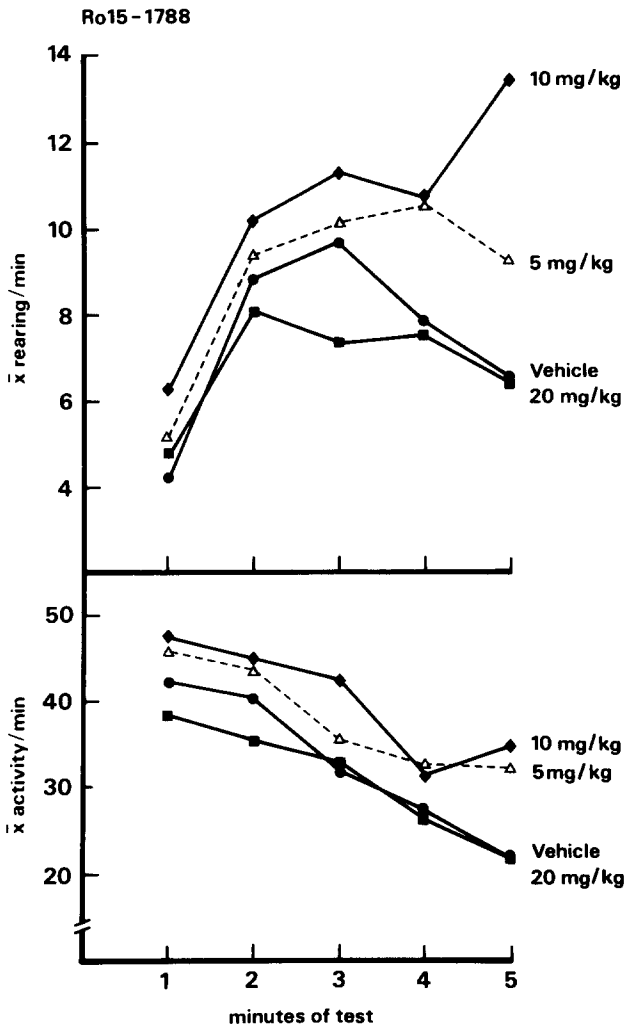

FIG. 1. Effects of Ro15-1788 (0–20 mg/kg, IP) on locomotor activity and rearing in male mice exposed to a novel environment. Analysis revealed that 10 mg/kg induced an increase in total rearing ($p<0.01$), whilst 5 and 10 mg/kg significantly altered the pattern of rearing over the test session ($p<0.01$). See text for details.

as the test arena, the floor of which was clearly divided into 20 equal squares. The arena was illuminated from above (2×60 W white bulbs) and visualized, by video-relay, on a TV monitor in an adjacent room. A multichannel event recorder was used to score locomotor activity (squares entered) and rearing on a minute-by-minute basis over a 5 min test session. Total defaecation for each subject was also noted and the arena thoroughly cleaned following each session. The combination of bright illumination, phase of testing (dark phase) and brief test period was chosen to enhance novelty.

Animals were randomly allocated to four treatment conditions (n=17):–vehicle, 5, 10 or 20 mg/kg Ro15-1788. The antagonist was suspended in distilled water to which two drops of Tween 80 were added. The vehicle consisted of a water/Tween mixture. All injections were performed in a volume of 10 ml/kg (IP), 20 min before testing. In an order counterbalanced for treatment condition, animals were individually placed in the arena and their behaviour recorded

TABLE 1

EFFECTS OF Ro15-17880 (0–20 mg/kg, IP) ON DEFECATION IN MALE MICE EXPOSED TO A NOVEL ENVIRONMENT

	Ro15-1788 (mg/kg)			
	Vehicle	5.0	10.0	20.0
Defecation	1(0–2)	2(1–3)	2(1–2.5)	1(0–1.5)

Data are expressed as medians (lower-upper quartiles). See text for details.

for 5 min. Experimenters remained blind to treatment conditions throughout the testing phase.

RESULTS

Locomotor Activity and Rearing

See Fig. 1. Data were initially subjected to 2-factor analyses of variance (ANOVA; drug condition and time period, repeated measures on the second factor). For locomotor activity, ANOVA failed to reveal either a significant main effect for drug, $F(3,64)=2.49$, NS, or a significant drug × time period interaction $(12,256)=0.72$, NS. Time period was significant, $F(4,256)=28.23$, $p<0.01$, reflecting intrasession habituation of this measure. In contrast, for rearing, ANOVA indicated a significant main effect for drug, $F(3,64)=5.07$, $p<0.01$, a significant drug × time period interaction, $F(12,256)=1.95$, $p<0.05$ and a significant main effect for time period, $F(4,256)=20.36$, $p<0.01$.

The main drug effect was further examined using Dunnett's tests (control vs. each drug condition), which indicated that 10 mg/kg Ro15-1788 significantly enhanced total rearing during the test session, $t(64)=3.01$, $p<0.01$. The significant interaction term was further studied by trend analysis. This procedure revealed significant linear trends towards increased rearing in groups treated with 5 mg/kg, $F(1,256)=13.08$, $p<0.01$ and 10 mg/kg, $F(1,256)=33.26$, $p<0.01$, Ro15-1788. Such trends were not apparent in either the vehicle, $F(1,256)=2.21$, NS, or 20 mg/kg Ro15-1788, $F(1,256)=1.21$, NS, groups.

Defaecation

See Table 1. Although, compared to vehicle-treated animals, defaecation was higher in groups receiving Ro15-1788 (5 and 10 mg/kg), statistical analysis failed to demonstrate significance (Kruskal-Wallis; $H(3)=7.35$, NS).

DISCUSSION

Although the imidazobenzodiazepine derivative, Ro15-1788, is known to antagonize many centrally-mediated effects of benzodiazepines, it has widely been reported to be devoid of intrinsic activity at low doses [3, 6, 16, 18, 20, 24, 28, 29]. However, current results (together with several other recent findings) are inconsistent with the latter proposition.

Firstly, when male mice are briefly exposed to a novel environment, Ro15-1788 significantly enhances rearing behaviour (10 mg/kg) and alters the pattern of this response over the test session (5–10 mg/kg). A similar, though non-significant, trend was observed on total locomotor activity (see Fig. 1). As rearing may be considered (at a descriptive

level) to be an index of exploration [1], these data are in general agreement with those of File *et al.* [12]. They found that, in male rats, Ro15-1788 (4–20 mg/kg) enhances exploratory head-dipping in the hole-board test, without significantly altering locomotor behaviour. In a further study, the same authors reported that Ro15-1788 (10 mg/kg) induces a weak anxiogenic action in the social interaction test of anxiety [11]. The present data indicate that in male mice, as well as rats, low doses of this benzodiazepine antagonist display significant intrinsic activity. Whilst no firm conclusion can be made regarding the nature of the observed effects, the pattern of disrupted habituation is not inconsistent with an anxiogenic effect. These observations may, in turn, reflect antagonism of a benzodiazepine-like substance which is released only under certain circumstances, e.g. in response to environmental novelty.

Secondly, our data also demonstrate that, with a higher dose (20 mg/kg), the positive behavioural effects of Ro15-1788 are no longer apparent, i.e., the behaviour of animals treated with this dose does not significantly differ from that of vehicle controls. Although this finding is not in agreement with File *et al.* [12], who reported a dose-dependent (4–20 mg/kg) enhancement of head-dipping in rats, it is consistent with their observation that the weak anxiogenic action of this compound is lost at 20 mg/kg [11]. Several other findings also bear on this issue:— at high doses (≥50 mg/kg), Ro15-1788 displays *anti*convulsant activity [21], *anti*aversive effects in a brain-stimulation escape task [17] and, in a drug discrimination test, weak benzodiazepine-appropriate responding [14]. This profile suggests that, at high doses, Ro15-1788 may be a partial agonist at benzodiazepine receptors—an action which may account for the loss of behavioural activity at the highest dose used in the current study.

In conclusion, the present findings represent the first demonstration of low-dose intrinsic behavioural activity of Ro15-1788 in mice. The pharmacological profile obtained suggests that (under present conditions), at doses of ≥20 mg/kg, this compound may act as a partial agonist. Further studies are currently underway to characterize the nature of Ro15-1788-induced behavioural changes in both non-social and social contexts.

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