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RO 15-1788 does not influence postpartum aggression in lactating female rats

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Abstract. Recently, Hansen et al. (1985) suggested behavioural similarities between lactating rats and non-maternal rats treated with benzodiazepines (BDZ), indicating that lactation may be associated with an increased activity state at the GABA/BDZ receptor complex similar to BDZ treatment. A logical prediction of this hypothesis is that BDZ antagonists should decrease typical maternal behaviours involved, such as aggression.

We tested this hypothesis by measuring the behavioural effects of the BDZ antagonist RO 15-1788 (1.25–10 mg/kg IP) on aggressive behaviour of lactating female rats confronted with male intruders. We could not support the hypothesis; no consistent behavioural effects of RO 15-1788 on aggression were found. The implications of this finding for the proposed hypothesis are discussed.

Key words: Benzodiazepines – Aggression – Lactation – Maternal behaviour – RO 15-1788 – Rat

In a recent paper attention was drawn to the similarity between the behaviour of lactating rats and benzodiazepine-treated non-maternal females (Hansen et al. 1985). Three characteristics of female rats during the postpartum period, i.e. increased food consumption, increased aggression towards conspecifics and a decrease in "freezing" in response to audiogenic stimulation, are features that are also suggested to occur during treatment of non-maternal females with low doses of benzodiazepines (Hansen et al. 1985). The latter authors therefore speculated that lactation might be associated with an increased activity at the GABA/benzodiazepine receptor complex (enhanced GABA-ergic neurotransmission) in certain brain areas. They tested this hypothesis in maternal rats using three benzodiazepine antagonists that are known to functionally antagonize some of the benzodiazepine effects, but are not devoid of intrinsic action (Haefely 1983). Aggression and food intake were decreased, whereas fear was increased by these drugs.

Although the endogenous GABA/benzodiazepine receptor complex may be changed during lactation, we recently found that maternal females with an already high basic aggression level are still sensitive to the stimulatory effects of low doses of chlordiazepoxide (CDP) on aggression (Olivier et al. 1985). We have not performed such studies on non-maternal females, but experiments in male rats showed a similar effect of CDP on male aggression (Olivier and Van Dalen 1982). This may suggest that the maternal state is not per se associated with a changed BDZ receptor state. Nevertheless, the hypothesis of an increased activity at the BDZ receptor site in lactating females is a very attractive one and we were interested in whether treatment with the pure benzodiazepine antagonist RO 15-1788 was able to affect maternal aggression in rats. If lactation indeed induces a state at the GABA/BDZ receptor comparable to a state induced by BDZ agonists (e.g. chlordiazepoxide) then it may be expected that treatment of lactating females with a pure BDZ antagonist like RO 15-1788 reduced aggression, at least at low doses where it exerts its full antagonistic activity. At higher doses, where RO 15-1788 may exert some agonistic activity (Pellow and File 1984), aggression should either not be affected or might even be enhanced.

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Material and methods

Fifteen Wistar females of 250–350 g, obtained from CPB-TNO, Zeist, were used. After mating, the primiparous females were placed in an observation cage (40 × 30 × 30 cm), where they stayed for the rest of the experiment. In this cage nesting material, food and water were freely available. The cages were placed in the observation room with a reversed day-night rhythm, night starting at 07.00 hours and ending at 19.00 hours. Tests were performed during the first part of the dark period (8.30–12.30 hours) under dim red light conditions. Pre-tests for aggressive responses of the females were done on day 2. Two animals were discarded because they showed no aggression on this day.

Aggression tests were performed on alternating days between day 4 and 12 postpartum; day of birth was regarded as day 0. The females were given five doses according to a balanced randomized block design and dosing days were alternated by wash-out days. RO 15-1788 (1.25, 2.5, 5 and 10 mg/kg; Hoffman-La Roche, Basel, Switzerland) was suspended in gelatin-mannitol (vehicle) and injected IP 30 min before testing in a volume of 2 ml/kg body weight. Tests consisted of a 5-min observation period during which a naive male, group-housed intruder (180–220 g) was present in the female's cage. The behaviour was videotaped and analysed later. Each intruder was used only once.

The behaviour of the females was scored using an ethogram previously described (Olivier et al. 1985). Seven categories were distinguished: Aggressive behaviour comprised bite attacks on head or body, lateral threat, upright posture,

Table 1. Effect of RO 15-1788 on female behaviour in a maternal aggression test against male intruders

	Dose (mg/kg IP)					χ^2	P
	0	1.25	2.5	5	10 mg/kg		
Aggression	33.4 (17.4–75.3)	36.4 (25.3–72.2)	45.1 (15.7–83.0)	42.6 (25.6–52.4)	34.0 (24.3–44.9)	5.11	0.28
ISB	80.4 (54.2–101.7)	64.9 (55.0–82.3)	73.0 (69.5–87.0)	99.2 (62.3–116.3)	102.5 71.3–124.1)	9.05	0.059
Exploration	117.3 (87.1–163.1)	109.7 (91.5–117.9)	114.7 (90.8–130.2)	87.6 (66.7–121.7)	99.6 (79.7–122.8)	7.2	0.13
Self care	13.1 (10.2–31.1)	24.7 (11.4–27.3)	14.5 (6.1–18.8)	13.1 (9.1–27.2)	17.0 (12.3–30.3)	4.12	0.39
Pup care	11.6 (3.4–27.1)	11.1 (8.9–48.0)	21.6 (4.1–46.4)	13.5 (4.0–41.0)	9.9 (2.9–21.8)	5.05	0.28
Avoidance	2.2 (1.0–4.0)	3.4 (1.6–8.7)	3.3 (1.8–7.0)	5.0 (0.8–8.5)	2.4 (1.1–7.4)	2.29	0.68
Inactivity	4.9 (1.4–9.5)	5.6 (1.3–12.3)	3.1 (2.3–17.2)	3.5 (0–10.0)	7.7 (4.7–16.6)	4.17	0.38

Median duration (and interquantile ranges) of the various behavioural categories in 5-min aggression tests as displayed by the lactating females. Friedman χ^2 and P-values are given in a separate column. Five doses were given IP, 30 min before testing

teeth chattering, nipping, pulling, clinch fights, kicking, lunge, jump attack and on top.
Introductory Social Behaviour (ISB) comprised moving towards the partner, sniffing of intruder, genital sniffing, social grooming and crawl under.
Attention, sniffing, rearing, locomotion, marking, digging and carrying of food particles belonged to the category Exploration.
Inactivity was made up of sitting and lying.
Avoidance was represented by moving away from the partner and keeping off the partner.
Pup care involved staying on the nest, including nursing elements like picking up and carrying the pups. Grooming, washing and shaking formed the last category, Self Care.

All data were analyzed by Friedman analysis of variance followed by multiple comparison tests (Conover 1980).

Results

Table 1 summarizes the results of RO 15-1788 treatment on the behaviour of lactating female rats. The mean durations of the seven behavioural categories did not change significantly. A non-significant effect was present in ISB. This was caused by the low level of social behaviour at the 1.25 mg/kg dose. All other behaviours did not change after drug treatment; the somewhat lower aggression level at 10 mg/kg was not significant.

The data for the frequencies of the behavioural categories show a similar picture (data not shown). In addition, the latencies of the first attack did not change after RO 15–1788 treatment.

Discussion

In the present experiment, the benzodiazepine receptor antagonist RO 15-1788 had no clear effects on aggressive behaviour of lactating female rats nor on any other behavioural category. RO 15-1788 is able to antagonize the behavioural effects of benzodiazepine agonists and inverse agonists like β-carbolines (Pellow and File 1984). If, according to the hypothesis of Hansen et al. (1985), the BDZ-GABA receptor complex in lactating female rats is activated in an essentially normal way after BDZ agonistic treatment in non-lactating females, our expectation was that RO 15-1788 should antagonize the aggressive behaviour of lactating females, which was one of the key behaviours in their hypothesis. RO 15-1788 had no such effect in lactating females in the present experiment. The doses of RO 15-1788 used are clearly in the normal range for antagonizing behaviours induced by BDZ agonists such as feeding or drinking (Cooper 1985 and own unpublished work). Therefore it seems plausible from our experiments that there is no enhanced activity state at the BDZ-GABA receptor complex in lactating female rats comparable to BDZ-treated virgin females.

Hansen et al. (1985) proposed their hypothesis of enhanced activity of the BDZ-GABA receptor complex in lactating female rats on the basis of behavioural similarities between BDZ-treated animals and lactating females. Three "functional" BDZ antagonists (FG 7142, pentylenetetrazol and caffeine) were able to decrease food intake and aggression and to enhance freezing in lactating female rats. In the view of Hansen et al. these findings support the hypothesis of enhanced GABA neurotransmission in motherhood. However, FG 7142 (a β-carboline) is an inverse agonist with marked intrinsic activity (Haefely 1983; Pellow and File 1984) and its behavioural effect in the experiment of Hansen et al. is no proof for their hypothesis. Similar arguments can be used both for pentylenetetrazol and caffeine, neither of which binds to the BDZ receptor itself (Haefely 1983; Pellow and File 1984) but both have behavioural actions of their own. Moreover, the use of only one dose of each drug in lactating females by Hansen et al. (1985) may have complicated their interpretation. As long as no dose-response studies and controls for specificity of the behavioural effects are included, the suggested similarities between mother rats and BDZ-treated non-maternal females may be disputed.

In contrast, RO 15-1788 has been suggested to be a behaviourally inactive antagonist at central benzodiazepine

receptor sites, a contention that cannot always be held in view of recent evidence that suggests that it has several intrinsic actions (File et al. 1982; De Vrij and Slangen 1985). At low doses (below 10 mg/kg) RO 15-1788 exerted anxiogenic effects in the social interaction test (see review by Pellow and File 1984). At higher doses (Pellow and File 1984) RO 15-1788 exerts benzodiazepine agonistic activity.

Although the hypothesis of a changed activity state at the BDZ/GABA receptor complex during motherhood in female rats sounds a very attractive way for explaining the observed behavioural changes during lactation, the present data on RO 15-1788 does not support this idea.

The dose range used (1.25–10 mg/kg) is sufficiently wide to exclude errors due to differential actions at different doses, and low enough not to enter the area at which (partial) agonistic actions may occur. Based on our observations, we suggest that motherhood in rats does not coincide with changes in the activity of the BDZ/GABA receptor complex. The behavioural similarities between BDZ-treated and lactating females may be coincidental and the effects of the "functional" BDZ antagonists used by Hansen et al. (1985) form no solid support for their hypothesis, as is also indicated by our data of RO 15-1788 on aggression of lactating female rats. The latter also indicates that RO 15-1788 is a neutral benzodiazepine antagonist in the present experiment. This is also supported in other models of aggression, where RO 15-1788 had limited effects on aggression and certainly no clearcut suppressive action (Rodgers and Waters 1984; Skolnick et al. 1985). Ideally, RO 15-1788 should also have been tested on non-lactating females in order to compare the behavioural effects in animals which, according to Hansen et al.'s hypothesis have a different state of activity of the BDZ/GABA receptor complex. Since non-lactating females are hardly aggressive such control experiments were not feasible. Indirect evidence against the enhanced activity state of the GABA/BDZ receptor complex is derived from our previous observations (Oliver et al. 1985, 1986), which showed that aggression of lactating females can still be enhanced by CDP treatment. If the lactating female is aggressive because of an (endogenous) activation of the benzodiazepine receptor, it is at least surprising that agonistic drugs like CDP can even increase this enhanced baseline level. The present experiments focus on the ability of RO 15-1788 to antagonize the CDP-induced increase of aggression.

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