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Effects of Long-Term Administration of Clozapine on Body Weight and Food Intake in Rats

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BAPTISTA, T., A. MATA, L. TENEUD, M. DE QUIJADA, H.-W. HAN AND L. HERNÁNDEZ. *Effects of long-term administration of clozapine on body weight and food intake in rats.* PHARMACOL. BIOCHEM. BEHAV. **45**(1) 51-54, 1993. — Previous reports have shown that long-term administration of typical and atypical neuroleptics induced obesity in female but not in male rats. It has been suggested that impaired ovarian steroidogenesis related to neuroleptic-induced hyperprolactinemia is necessary to observe the body weight changes. This hypothesis was tested with clozapine, an atypical neuroleptic that produces in rats a shorter increase in serum prolactin levels than do other neuroleptics. The effects of clozapine on body weight and food intake were assessed in female and male rats under treatment with any of the following doses: 0.5, 1, 2.5, 5, 10, and 20 mg/kg IP for 21 days. Vaginal cycle under clozapine treatment, as an indirect indicator of ovarian steroidogenesis, was also assessed. Obesity was not observed in any group. By contrast, clozapine at the doses of 10 and 20 mg/kg significantly decreased body weight and feeding in male rats. Clozapine at the doses of 5 and 10 mg/kg IP induced permanent diestrus. The failure of clozapine to induce obesity in female rats, despite impaired vaginal cycle, can be considered indirect evidence that drug-induced hyperprolactinemia is not sufficient to observe neuroleptic-induced obesity in rats.

Obesity Rats Neuroleptics Clozapine

ANTIPSYCHOTIC drugs exert profound effects on body weight and feeding in humans (24) and rats (1).

Chronic administration of thioridazine, trifluoperazine, sulpiride, and haloperidol significantly increase body weight in female but not in male rats (1,2). This phenomenon has been studied in depth with sulpiride, a specific D_2 dopamine receptors blocker (12).

At least two different mechanisms might mediate sulpiride effects on body weight and feeding in rats: a) a neuronal mechanism in the perifornical hypothalamus, where a dopaminergic satiety system has been postulated (13). Sulpiride could block D_2 receptors and disinhibit feeding neurons in that hypothalamic area (1, 3). b) A hormonal mechanism involving the gonadal steroids. Sulpiride-induced hyperprolactinemia that is mediated by the blockade of D_2 receptors in the pituitary might impair ovarian steroidogenesis and cause rats to increase their food intake (20,21).

There is evidence to support the existence of a neuronal mechanism. For example, direct injections of sulpiride in the perifornical hypothalamus induce feeding and drinking (19) and drive masticatory neurons in rats (17). In addition, systemic sulpiride increases dopamine metabolites in the lateral hypothalamus (3). This fact probably reflects blockade of D_2 receptors in that area.

Likewise, the existence of a hormonal mechanism is supported by the following facts: the prevention of sulpiride-induced body weight gain by concomitant administration of estradiol, the absence of an additional body weight increase after sulpiride treatment in previously ovariectomized rats, and the induction of permanent diestrus in sulpiride-treated rats, which suggests an impairment of ovarian steroidogenesis (20).

The main assumption of the hormonal mechanism is that drug-induced hyperprolactinemia indirectly increases food intake (20). In fact, all the neuroleptic drugs tested through the present in long-term experiments induce considerable increase in serum prolactin levels (9).

Clozapine is an antipsychotic agent that displays atypical pharmacological and clinical properties in relation to the classic antipsychotics (7). Clozapine has a relatively weak central dopaminergic activity (7) and affects serum prolactin levels in a different manner compared to the other neuroleptics. In rats, clozapine produces rapid elevations in serum prolactin levels similar in magnitude to those produced by haloperidol,

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but the prolactin response is of much shorter duration than that elicited by haloperidol (10). A similar pattern has been observed in humans treated with clozapine (16). These studies show that subjects under a single-dose schedule of clozapine treatment display normal serum prolactin levels most of the day. However, it is unknown if this short increase in serum prolactin is enough to impair gonadal steroidogenesis.

The present article describes the effects of clozapine on body weight (BW) and food intake (FI) in female and male rats. In addition, ovarian function under clozapine treatment was indirectly assessed by monitoring cyclic vaginal cornification. Despite altered vaginal cycle, clozapine did not induce obesity in female rats. BW and FI were significantly reduced in male rats.

METHOD

Animals

Wistar male rats weighing between 250–280 g and females weighing between 220–250 g were individually housed in a 12 L : 12 D cycle. Animals received a high-fat diet (66% powdered rat food and 33% corn oil) and water ad lib. Food was placed in spillage-proof feeders.

Drug Administration

Clozapine (Sandoz Research Institute, East Hanover, NJ) was dissolved in 0.1 N HCl and adjusted to pH 6 with 0.1 N NaOH. For each sex, there were seven groups of rats: One group received vehicle IP and the other groups received any of the following doses of clozapine—0.5, 1, 2.5, 5, 10, or 20 mg/kg IP. For female rats, each group had 10 rats; for male rats, each group had 6 rats. Treatments were administered during 21 days as a single daily injection at 0900 h.

Body Weight and Food Intake Measurement

Body weight and feeding were measured daily at 0800 h, beginning 1 week prior to the treatments. For each animal, BW gain was calculated by subtracting BW when starting treatment from the BW at the end of injections. For every sex, BW gain and FI data of rats under clozapine and vehicle were compared by a two-way analysis of variance (ANOVA) followed by the Newman-Keuls test.

Vaginal Cycle Assessment

Vaginal smears were taken daily between 1100 and 1300 h. Rats that showed at least four consecutive 4-day estrous cycle were selected. Four groups were formed: One received daily injections of vehicle IP and the others received one of the following doses of clozapine—2.5, 5, and 10 mg/kg IP during 14 days. Cytological assessment was conducted with light microscopy. Data were analyzed with a χ^2 test followed by Ryan's test (14).

RESULTS

Body Weight and Food Intake

BW gain during clozapine treatment was not significantly affected in female rats; however, at the dose of 10 and 20 mg/kg clozapine significantly reduced BW in male rats (Fig. 1).

FI during clozapine treatment was not significantly affected in female rats (data not shown), but clozapine at the doses of 10 and 20 mg/kg significantly decreased FI in male rats (Fig. 2).

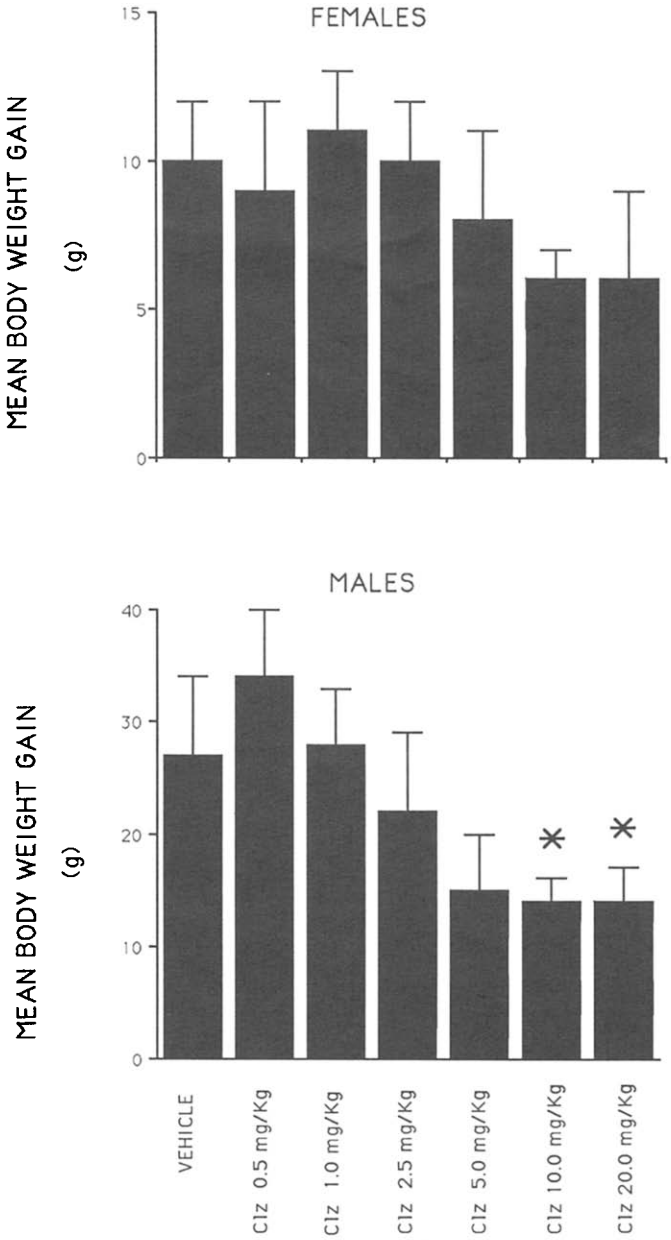

FIG. 1. Body weight gain of female rats and male rats treated with clozapine or vehicle IP for 21 days. Values represent mean ± SEM. No significant effect was observed in female rats, $F(6, 54) = 1.4$, $p < 0.1$. Clozapine at doses of 10 and 20 mg/kg significantly decreased body weight gain in male rats, $F(6, 30) = 5.6$. *$p < 0.001$.

Vaginal Cycle

All rats treated with vehicle maintained a normal vaginal cycle, that is, they displayed successive cytological changes of proestrous, estrous, and diestrous during all the period of

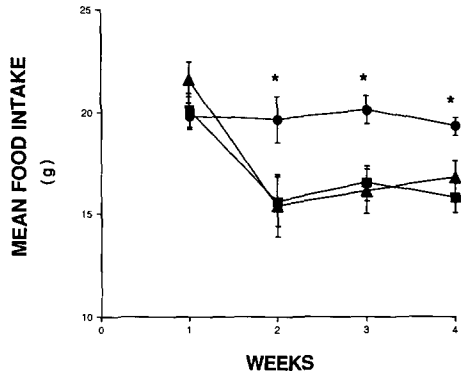

FIG. 2. Food intake of male rats treated with clozapine 10 (■) and 20 (▲) mg/kg IP or vehicle (●). Values represent mean ± SEM. After 1 week of control, treatments were administered during 3 weeks. Both doses of clozapine significantly decreased food intake, $F(22, 162) = 22.5$. $*p < 0.001$.

injections. Clozapine at the doses of 5 and 10 mg/kg significantly decreased the number of animals that kept a normal vaginal cycle, that is, some of them remained in permanent diestrous during the treatment (Table 1).

DISCUSSION

Previous reports have shown that long-term administration of typical (haloperidol and trifluoperazine) and atypical (thioridazine and sulpiride) neuroleptic drugs increase BW in female but not in male rats (1,2,20).

Chronic administration of a wide range of clozapine doses did not cause significant changes in BW in female rats and decreased BW and FI in male rats.

These results are of clinical and pharmacological interest. Obesity is a troublesome adverse effect of neuroleptics in humans, and it diminishes compliance in long-term treatments (8,22–24). Therefore, if it were shown that clozapine induces less obesity in humans than do other neuroleptics its use in obesity-prone patients should be encouraged. Reports have shown that BW gain is observed during long-term clozapine administration (6,7). However, the number of patients under follow-up was small, and it deserves further investigation.

Obesity related to chronic neuroleptics administration is observed in adult female but not in male rats (1,2). This trend was also observed with clozapine. The effect of age and sex on neuroleptics-induced obesity in humans has not been explored. This is an important point because it could be hypothesized that, as it happens in rats, women might be more prone than men to gain weight under neuroleptic treatment. Sex differences in weight gain under other psychotropic drugs, such as lithium, has also been observed in humans and rats (4). Therefore, the influence of sex on BW gain in humans under neuroleptic treatment warrants further clarification. Again, if the effect of clozapine on BW observed in rats is also found in humans its use should be encouraged when obesity is a clinical problem. However, research in humans is complicated by several factors, for example, mental illnesses and polydrug use.

From the pharmacological perspective, clozapine differs from other neuroleptics in several ways; it is a relatively weak antagonist at striatal D_2 dopamine receptors and produces a more potent blockade of central dopamine D_1, cholinergic, serotonergic S_2, histamine H_1, and α_1- and α_2-adrenergic receptors (7). In addition, clozapine-induced hyperprolactinemia is smaller and shorter than the one produced by other neuroleptics (7,10,16).

Two mechanisms (neural and neuroendocrine) have been postulated to explain neuroleptic-induced obesity in rats (1,3,20). The neural mechanism postulates that blockade of dopaminergic neurons in the lateral hypothalamus disinhibits feeding (1,3). The endocrine mechanism states that neuroleptic-induced hyperprolactinemia impairs ovarian steroidogenesis and decreases serum estrogen levels. The reduction in estrogen levels could render hypoactive satiety neurons in the ventromedial hypothalamus (VMH) (21).

The role of the VMH is supported by the following facts: Estrogen receptors have been identified on VMH cells (15), estradiol implantation in this region reduces BW and FI in previously ovariectomized rats (11), and VMH lesions considerably attenuate the obesity and hyperphagia induced by gonadectomy in female rats (5). Therefore, hyperprolactinemia might be a necessary step in neuroleptic-induced obesity in rats. In the present experiments, ovarian function was indirectly assessed through the vaginal cycle. Three doses of clozapine were tested: 2.5, 5, and 10 mg/kg. The two highest doses suppressed the vaginal cycle, suggesting impairment of ovarian steroidogenesis, but these doses of clozapine did not induce obesity in rats. Therefore, these results suggest that impaired ovarian steroidogenesis related to increase in serum prolactin levels is not sufficient to induce obesity in female rats.

Hyperprolactinemia is related to blockade of D_2 dopamine

TABLE 1

EFFECT OF CLOZAPINE AND VEHICLE ADMINISTRATION ON THE VAGINAL CYCLE IN RATS

Treatment	n	Rats With Normal Vaginal Cycle	Rats With Permanent Diestrus	χ^2
Vehicle	10	10	0	
Clz 2.5 mg/kg	10	8	2	0.55 (NS)
Clz 5 mg/kg	10	3	7	7.9*
Clz 10 mg/kg	10	0	10	16.2*

n, number of subjects per group; Clz, clozapine; NS, nonsignificant. The overall χ^2 test showed the following values: $\chi^2(3) = 43$; $p < 0.0001$.

Ryan's test; $p < 0.01$ with respect to the vehicle group.

receptors in the pituitary (9). Compared to other neuroleptics, clozapine is a weak antagonist at striatal D_2 receptors (7). In addition, clozapine is a more potent blocker than other neuroleptics of D_1, cholinergic, serotonergic, histaminergic, and adrenergic receptors (7). The role of these receptors in the differential effect of clozapine on BW and FI in female rats cannot be discarded and deserves further exploration.

Regarding male rats, previous reports have shown that several types of neuroleptics tend to induce a nonsignificant decrease in BW (1). Neuroleptics decrease serum testosterone levels (18), which should decrease BW. In fact, all the neuroleptics tested through the present induce a nonsignificant decrease in BW. The highest dose of clozapine induced a significant decrease in BW and FI in males; however, it appears as a toxic effect. The fact that females gain BW whereas male rats lose BW has also been observed with long-term administration of lithium (4). Therefore, male rats appear to be more sensitive than females to the toxic effects of clozapine and lithium.

In summary, a wide range of clozapine doses did not increase BW and FI in rats. This finding contrasts with the effects of other atypical neuroleptics such as thioridazine and sulpiride, which induce obesity in female rats (1). These results could be considered as indirect evidence that hyperprolactinemia is not the only factor necessary to observe neuroleptic-induced obesity in rats.

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