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Enhancement of Amphetamine Anorexia After Chronic Administration of Sulpiride in Rats

TRINO BAPTISTA,[1] LUIS TENEUD AND LUIS HERNÁNDEZ

Laboratorio de Fisiología de la Conducta, Facultad de Medicina, Universidad de Los Andes, Mérida 5101-A, Venezuela

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BAPTISTA, T., L. TENEUD AND L. HERNÁNDEZ. *Enhancement of amphetamine anorexia after chronic administration of sulpiride in rats.* PHARMACOL BIOCHEM BEHAV **45**(1) 45–49, 1993.—Long-term administration of sulpiride induces hyperphagia and obesity in female rats. After sulpiride withdrawal, a significant hypophagia has been observed. The hyperphagia could be related to the blockade and the hypophagia to supersensitivity of dopamine D_2 receptors, in particular those D_2 receptors located in the perifornical hypothalamus. If this were the case, an enhancement of anorexia induced by amphetamine and dopamine should be observed after interruption of long-term sulpiride treatment. Two doses of systemic sulpiride (20 or 200 mg/kg) and one dose of intrahypothalamic sulpiride (15 μg) were tested. Amphetamine was administered by systemic or intrahypothalamic infusion. Dopamine was administered in the hypothalamus. After withdrawal of systemic administration of sulpiride (200 mg/kg), an enhancement of anorexia induced by systemic amphetamine was observed. However, the anorexia induced by intrahypothalamic injections of amphetamine or dopamine was not affected by the interruption of the sulpiride treatment. These results suggest that the hypophagia following chronic sulpiride treatment is not due to supersensitivity of D_2 dopamine receptors in the lateral hypothalamus. Moreover, the change in the response to amphetamine might be related to supersensitivity of extrahypothalamic D_2 receptors.

Sulpiride Anorexia Dopamine receptors supersensitivity Amphetamine Dopamine Perifornical hypothalamus Rats

SULPIRIDE is a benzamide that has been used to prevent vomiting and relieve the symptoms of schizophrenia (17,26). Both effects have been attributed to a blockade of dopamine receptors (26). Specifically, sulpiride binds strongly to D_2 receptors (14). Chronic sulpiride treatment increases body weight in psychiatric patients (2) and rats (3), specifically in female rats and prepuberal males (4).

Blockade of D_2 receptors by sulpiride and body weight increase might be causally related. The crucial D_2 receptors that sulpiride blocks to induce obesity might be located in the perifornical lateral hypothalamus (PFH), where a dopaminergic satiety system has been postulated (16). Several lines of evidence support the hypothesis that blockade of the dopaminergic satiety system in the PFH might cause hyperphagia and obesity during chronic systemic sulpiride treatment in rats (3,6,16,20–24).

However, other D_2 receptors might participate in sulpiride-induced obesity, for instance the D_2 receptors located in the anterior pituitary, which inhibit prolactin secretion (14). When the anterior pituitary D_2 receptors are blocked by sulpiride, hyperprolactinemia is induced. The rise in prolactin could cause hyperphagia and obesity because systemic administration of prolactin increases food intake and body weight in female rats (19). In addition, the D_2 receptors in the area postrema probably inhibit feeding at the end of a meal (10,17). Blockade of these receptors might enhance feeding. In fact, lesions of the area postrema increase eating of palatable food in rats (12).

Chronic sulpiride administration produces dopamine receptor supersensitivity (DRS), evidenced as an increase of striatal and mesolimbic binding sites (B_{max}) for ^{3}spiperone, $^3N,n$-propylnorapomorphine, and ^{3}sulpiride in rats treated with sulpiride for 3 weeks (9,13,25). Behaviorally, DRS appears as an exaggeration of behaviors induced by dopamine agonists (9,13,25). The interruption of chronic administration of sulpiride causes hypophagia and body weight lost (3). These effects are probably due to DRS (5). In the present study, we hypothesized that sulpiride withdrawal would enhance the anorexia induced by systemic administration of amphetamine. Further, if the crucial DRS occurs in the PFH then anorexia induced by intrahypothalamic injections of amphetamine or dopamine should be enhanced too. We found that after sulpir-

[1] Requests for reprints should be addressed to Trino Baptista, Apartado de Correos 93, Mérida, 5101-A, Venezuela.

ide withdrawal the anorexia induced by systemic administration of amphetamine was enhanced, but this was not the case with intrahypothalamic administration of amphetamine or dopamine.

METHOD

Subjects

Two-hundred and 27 adult, female rats of the Wistar strain weighing 200–250 g were individually housed with high-fat diet (66% powered rat food and 33% corn oil) and water ad lib on a 12 L : 12 D cycle. Food was placed in spillage-proof feeders. Body weight and food intake were measured daily.

Surgery

Under ketamine anesthesia (100 mg/kg, IP), bilateral 26-ga stainless steel guide cannulae aimed 2 mm above the perifornical hypothalamus were implanted. The stereotaxic coordinates were: 6.5 mm anterior to the interaural line, 1.6 mm lateral to the midsagittal sinus, and 5.7 mm ventral to the cortical surface. The injectors were 33-ga stainless steel tubes connected by PE 20 tubing to a 10-ml syringe mounted on a syringe pump. They protruded 2 mm off the tip of the guide shafts. The injection rate was 0.5 μl in 30 s.

Group Configuration

For intrahypothalamic injections, rats were chosen for the study based upon the exhibition of strong feeding or drinking behavior following acute administration of a bilateral injection of sulpiride in the PFH (15 μg/0.5 μl/30 s) at least 3 days before initiation of the study. One-hundred and 32 rats met these criteria. With this preliminary screening, it was reasonable to assume that injections of amphetamine or dopamine were acting on dopamine D_2 receptors.

Rats were divided into amphetamine intraperitoneal and intrahypothalamic and dopamine intrahypothalamic groups. The amphetamine/intraperitoneal group was then divided into sulpiride and vehicle subgroups, each having rats that received either 0.25, 0.5, or 1.0 mg/kg amphetamine prior to or following administration of sulpiride or vehicle. The amphetamine/intrahypothalamic group was also divided into sulpiride and vehicle subgroups, but received only one dose of amphetamine directly into the PFH (total dose of amphetamine was 30 μg/1 μl/30 s) (22). This one dose of amphetamine was also administered prior to or following sulpiride or vehicle injections.

The dopamine/intrahypothalamic group was comprised of sulpiride and vehicle subgroups, but received only one dose of dopamine in the PFH (total dose of dopamine was 20 μg/1 μl/30 s) (20). All the amphetamine or dopamine sulpiride groups were subdivided into those that received sulpiride (20 mg/kg, IP, for 21 days) and those receiving sulpiride (200 mg/kg, IP, for 21 days) (3,4,9,13,25). In addition, the anorectic effect of intrahypothalamic dopamine was assessed in rats treated with sulpiride directly injected into the PFH (total daily dose of sulpiride: 30 μg/l μl/30 s for 21 days) or the same volume of vehicle for 21 days.

Drugs

Racemic sulpiride (Delagrange, Paris, France) was dissolved in 0.1 N HCl. *d*-Amphetamine sulphate and pargyline (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% NaCl and dopamine (Sigma) was dissolved in 0.3% ascorbic acid. The dopamine experiments were conducted in pargyline-pretreated or pargyline-free rats. Pargyline is an inhibitor of monoamine oxidase tht prevents the rapid degradation of exogenous dopamine. It was used to potentiate the effect of dopamine on hypothalamic neurons because in our hands anorexia induced by local administration of dopamine in the PFH is not a robust phenomenon (see below for further discussion). In addition, a negative result in pargyline-free rats would be more reliable if it were negative in pargyline-pretreated rats too. Pargyline HCl (15 mg/kg, IP) was injected 0.5 h before the dopamine test.

Procedure

Basal conditions. During a period of 10 days before chronic sulpiride or vehicle administration, the effects of amphetamine or dopamine on food intake were assessed. Rats were food deprived for 24 h before the test. Drugs were administered at 0900 h. Each rat received four injections: two of amphetamine or dopamine and two of vehicle. Food intake was measured during 1 h after drug or vehicle administration. Injections were done 2 days apart in a counterbalanced order. The anorectic drug amphetamine was administered systemically or locally into the PFH. The anorectic effect of dopamine was only tested with intrahypothalamic administration. The amphetamine or dopamine anorectic effect was expressed as "percentage of reduction of food intake."

Sulpiride long-term administration and withdrawal. After completing the control tests, sulpiride or vehicle was administered daily for 21 days by intraperitoneal or intrahypothalamic routes (see group configuration). The tests of amphetamine- or dopamine-induced anorexia were repeated during the 10 days following sulpiride or vehicle withdrawal.

Statistical Analysis

The average of food intake under drug condition and under vehicle condition was calculated. Finally, a percentage of food intake under drug condition with respect to food intake under vehicle condition was calculated for each rat. This way, we obtained a figure that we called "percentage of reduction of food intake." The percentage of reduction of food intake after chronic treatment with sulpiride or vehicle was subtracted from the percentage of reduction of food intake before treatments. The resulting figure for each animal was defined as "change in the percentage of reduction of food intake." The changes in the percentage of reduction of food intake in the sulpiride-treated group and its vehicle-treated control group were compared by a one-way analysis of variance (ANOVA). The hypothesis tested here predicted that the amphetamine- or dopamine-induced anorexia should be greater after sulpiride withdrawal.

RESULTS

The cannulae tracks were located in the perifornical hypothalamus in the planes A5150-A5340 according to the Atlas of König and Klippel (15).

Amphetamine-induced Anorexia

Systemic amphetamine. No effect was observed in rats treated with sulpiride at the dose of 20 mg/kg IP, but in rats that received 200 mg/kg IP sulpiride for 21 days amphetamine at the doses of 0.5 and 1.0 mg/kg IP induced an anorexia that was significantly greater than in the vehicle-treated group. With amphetamine at the dose of 0.25 mg/kg IP, the same trend was observed but it reached only marginal significance due to a large interindividual variation (Fig. 1).

Intrahypothalamic amphetamine or dopamine. The anorexia induced by intrahypothalamic amphetamine was not

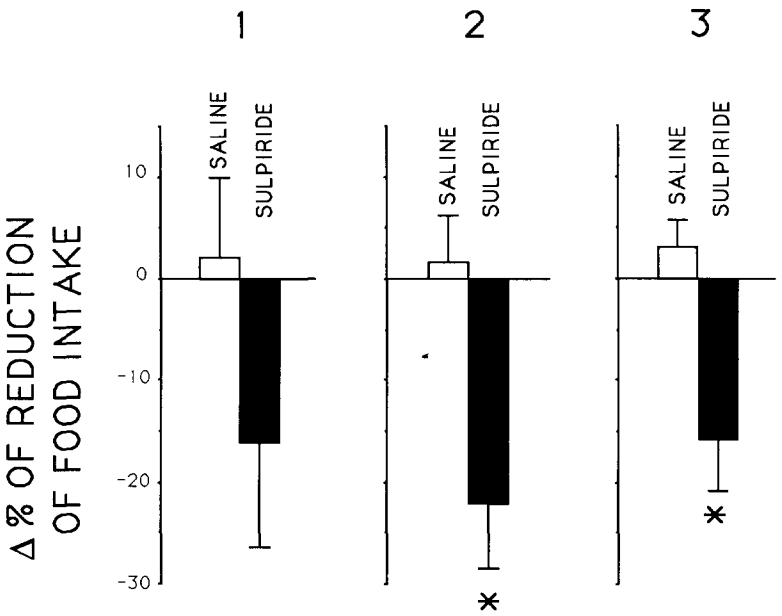

FIG. 1. Change in the percentage of reduction of food intake (with respect to vehicle) after systemic amphetamine in rats treated with daily injections of sulpiride (200 mg/kg, IP) for 21 days (black bars, $n = 7$) or NaCl 0.9% 0.3 cc IP for 21 days (open bars, $n = 7$). With the dose of amphetamine of 0.25 mg/kg IP (1) no significant effect was observed, $F(1, 12) = 1.96$, $p < 0.1$, but with the doses of 0.5 and 1 mg/kg IP (2 and 3, respectively) an enhancement of amphetamine anorexia was observed, $F(1, 12) = 9.23$, $p < 0.01$, and $F(1, 12) = 12$, $p < 0.004$, respectively.

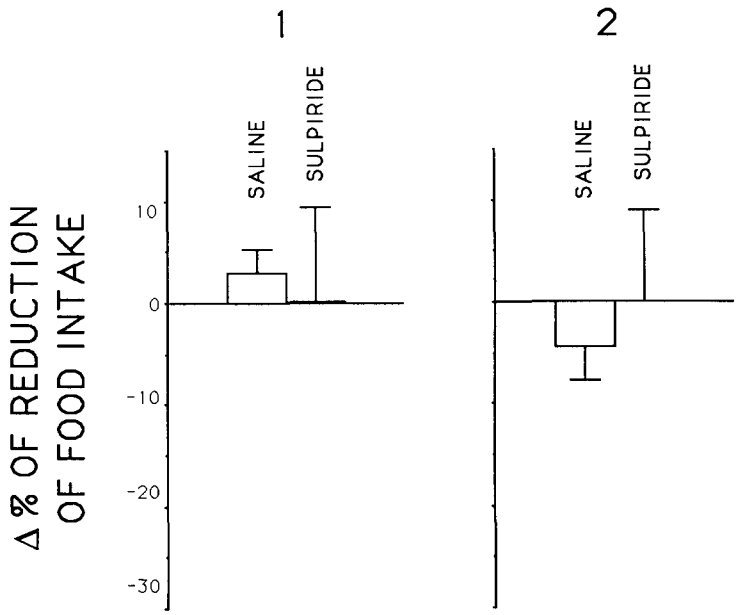

FIG. 2. Change in the percentage of reduction of food intake (with respect to vehicle) after intrahypothalamic dopamine in rats treated with sulpiride (200 mg/kg, IP) for 21 days (black bars, $n = 9$) or NaCl 0.9% 0.3 cc IP for 21 days (open bars, $n = 9$). Dopamine-induced anorexia was not significantly affected in rats pretreated (1) or not with pargyline (2), $F(1, 18) = 0.23$, $p < 0.6$, and $F(1, 18) = 0.08$, $p < 0.7$, respectively.

modified (data not shown). The anorexia induced by administration of dopamine into the PFH was not significantly changed in any group treated with systemic or intrahypothalamic sulpiride (Fig. 2).

DISCUSSION

After daily administration of sulpiride at the dose of 20 mg/kg IP, the anorectic effect of systemic amphetamine was not significantly different from the amphetamine effect in vehicle-treated rats. However, when the dose of sulpiride was 200 mg/kg IP the anorexia induced by systemic amphetamine was significantly enhanced. These results suggest that the hypophagia after sulpiride withdrawal was probably due to DRS. However, sulpiride withdrawal did not affect the anorexia induced by intrahypothalamic injections of amphetamine or dopamine. Therefore, the enhancement of amphetamine-induced anorexia at the withdrawal of sulpiride is probably due to DRS in extrahypothalamic areas.

Extrahypothalamic dopamine receptors involved in feeding regulation are located in the mesolimbic system, mesocortical system, the area postrema, and the nucleus of the tractus solitarius (10–12). Low doses of amphetamine injected into the nucleus accumbens increase feeding, but high doses of amphetamine decrease it (8). Therefore, if dopamine in the nucleus accumbens plays a predominantly inhibitory role in feeding DRS in this area could underlie the amphetamine anorexia enhancement. Such an anorexia enhancement does not have to be linked to satiety because amphetamine-induced hypermotility due to nigrostriatal and mesolimbic DRS could also disrupt feeding.

On the other hand, the D_2 receptors in the area postrema and the nucleus of the tractus solitarius participate in emesis (17). The activation of these receptors by apomorphine induces vomiting in mammals. Sulpiride withdrawal might cause DRS in the area postrema and in the nucleus of the tractus solitarius. In that case, systemic injections of amphetamine might release catecholamines in those areas and enhance anorexia. Considering the contribution of extrahypothalamic D_2 receptors to amphetamine anorexia, the dopamine receptors in the PFH could be necessary but not sufficient to inhibit feeding. Extrahypothalamic dopamine might act synergistically with D_2 receptors in the PFH or play a more important role in sulpiride withdrawal hypophagia.

Alternatively, neurotransmitters other than dopamine could be involved in the anorexic effect of amphetamine in the lateral hypothalamus (21). In fact, a good correlation between anorexia induced by intrahypothalamic injections of serotonin and amphetamine has been found (23). Therefore, the DRS in the hypothalamus could be masked by a decrease of amphetamine-induced release of serotonin. This explanation seems unlikely because neither systemic nor local administration of sulpiride enhanced the anorexigenic effect of dopamine in the PFH (Fig. 2).

Finally, the lack of enhancement of the anorexia induced by intrahypothalamic infusions of amphetamine and dopamine after sulpiride withdrawal suggests that hypothalamic D_2 receptors do not develop supersensitivity after long-term pharmacological blockade. It is well known that some dopamine receptors show supersensitivity and other do not. For example, DRS in the nigrostriatal and mesolimbic systems is achieved after chronic sulpiride or haloperidol administration (7,9,13,25). On the other hand, it is debatable as to whether or not pituitary D_2 dopamine receptors in rats develop DRS after pharmacological manipulations. Annunziato et al. (1) found that, after repeated neuroleptic treatment, the serum prolactin levels were significantly lower in rats treated with an acute dose of neuroleptics. This suggests DRS in the pituitary receptors. However, Dyer et al. (7) found that serum prolactin levels at the end of 1 year of neuroleptic treatment remained elevated compared to those in control animals, and the elevation of prolactin levels was not different from that obtained by administration of an equivalent acute single dose of neuroleptics. This is not consistent with the development of DRS in the pituitary receptors.

In conclusion, the results suggest that the hypophagia observed after sulpiride withdrawal and the enhancement of amphetamine anorexia might be due to dopamine receptors supersensitivity. These results also suggest that extrahypothalamic dopamine receptors might play an important role in the anorexia induced by amphetamine.

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