Pharmacology Biochemistry & Behavior, Vol. 37, pp. 227–229. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Systemic Sulpiride Increases Dopamine Metabolites in the Lateral Hypothalamus

TRINO BAPTISTA,* LUIS HERNANDEZ* AND BARTLEY G. HOEBEL†[1]

†Department of Psychology, Princeton University, Princeton, NJ 08544-1010
**Laboratorio de Fisiologia de la Conducta, Facultad de Medicina, Universidad de los Andes, Merida, Venezuela*

Received 10 November 1989

BAPTISTA, T., L. HERNANDEZ AND B. G. HOEBEL. *Systemic sulpiride increases dopamine metabolites in the lateral hypothalamus*. PHARMACOL BIOCHEM BEHAV 37(2) 227–229, 1990.—In rats with microdialysis probes in the perifornical lateral hypothalamus (PFH) a single injection of the D_2 receptor blocker l-sulpiride (20 mg/kg IP) significantly increased extracellular dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), but not 5-hydroxyindoleacetic acid (5-HIAA). This suggests that sulpiride crosses the blood-brain barrier and blocks D_2 dopamine receptors in the PFH leading to increased dopamine turnover reflected in increased extracellular DOPAC and HVA. We conclude that D_2 blockade in the hypothalamus could play a role in the hyperphagia and body weight gain observed in female rats under chronic administration of the antipsychotic drug, sulpiride.

Sulpiride Dopamine D_2 receptors Atypical neuroleptic obesity Rat Lateral hypothalamus Microdialysis

SYSTEMIC injections of sulpiride, a specific D_2 dopamine receptor blocker, increases food intake and body weight in female rats (2). An increment in body weight has also been observed in women receiving oral sulpiride (1). At least two different mechanisms might mediate this effect. One is a neural mechanism in the perifornical lateral hypothalamus (PFH) where a dopaminergic satiety system has been postulated (15). D_2 dopamine receptors are present in the PFH (21), and injections of sulpiride in the PFH induce feeding in satiated rats (18). Therefore, systemic injections of sulpiride could block D_2 receptors and thereby disinhibit feeding neurons in the PFH (2). The main argument against this interpretation stems from the suspicion that sulpiride penetrates the blood-brain barrier poorly. Autoradiographic studies have shown that systemically injected radioactive sulpiride labels the pituitary, but not the brain (4). However, other studies have suggested that 4-[^{3}H]sulpiride might be able to penetrate the blood-brain barrier (16). A second possible explanation for sulpiride-induced weight gain involves a hormonal mechanism. Indirect evidence suggests the involvement of gonadal hormones. There are sex- and age-dependent effects of systemic sulpiride on body weight and food intake in rats (2,3); sulpiride-induced weight gain in female rats is prevented by concomitant administration of estradiol, and there is an absence of body weight increase after sulpiride in previously ovariectomized rats (19). We have hypothesized that the sulpiride-induced hyperprolactinemia that is mediated by the blockade of D_2 dopamine receptors in the pituitary (8), might impair ovarian steroidogenesis (17) which, in turn, might cause the female rats to increase their food intake (19).

It is also possible that systemic sulpiride might be acting on both hypothalamic and pituitary receptors to induce hyperphagia and body weight gain. In the present experiment we attempted to find out if systemically injected sulpiride crosses the blood-brain barrier at the level of the PFH and blocks D_2 receptors. This experiment is based on the fact that in vitro and in vivo studies have shown that a temporary increase in DOPAC and HVA is correlated with increase in brain dopamine synthesis and turnover (5). Additionally, the blockade of postsynaptic dopamine receptors by a single dose of sulpiride or haloperidol increases extracellular DOPAC, HVA and dopamine in the striatum (9,13). The rationale is that if systemic sulpiride blocked D_2 dopamine receptors in the PFH an increase in DOPAC and HVA should be observed in this area. This hypothesis was tested using microdialysis in freely moving rats. The extracellular concentration of 5-HIAA was also assessed; dopamine levels were too low to detect.

Under general anesthesia with pentobarbital (20 mg/kg) and ketalar (40 mg/kg), 14 female Sprague-Dawley rats weighing between 230 and 250 g were implanted with 10 mm, 21-gauge guide cannulas aimed at the PFH. Stereotaxic coordinates for guide shafts were 6.5 mm anterior to the interaural line, 1.5 mm lateral to the midsagittal sinus and 3 mm ventral to the surface of the cortex. Animals recovered for at least 1 week following surgery before the experimental session. Technical details of the probe design are given elsewhere (10). In brief, probes were made with a concentric 36-gauge stainless steel tube inside a 26-gauge tube and a microdialysis tip of cellulose tubing 0.2 mm outside diameter, 3 mm long with a 6000 molecular weight cutoff. The probes were perfused at 1 μl/min through a swivel joint connected to a gas-tight syringe loaded with Ringer's solution of 189 mM NaCl, 3.9 mM KCl, and 3.37 mM $CaCl_2$ warmed at 37°C and

[1]Requests for reprints should be addressed to Dr. Bartley G. Hoebel.

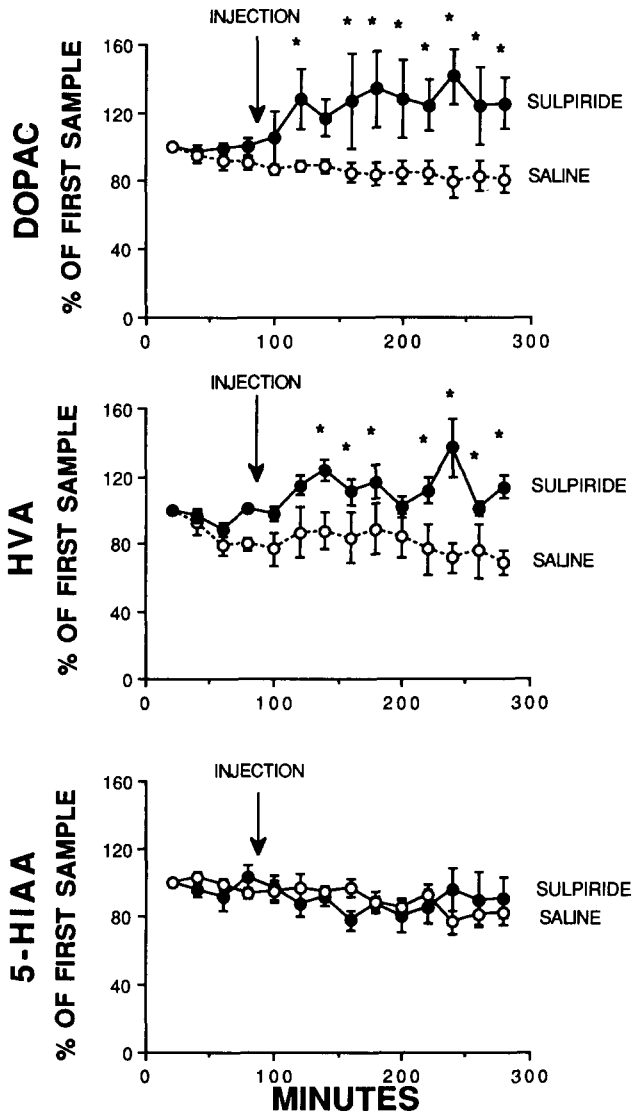

FIG. 1. Effect of a single dose of l-sulpiride (20 mg/kg/IP, filled circles, N = 7), or an isovolumetric dose of saline IP (open circles, N = 7) on the extracellular levels of DOPAC, HVA and 5-HIAA. Values represent percent with respect to the first baseline sample (mean ± SEM). Asterisks indicate significant differences. Systemic sulpiride caused DOPAC and HVA to increase significantly: $F(1,13) = 18$, $p<0.001$ and $F(1,13) = 19.2$, $p<0.001$, respectively; but 5-HIAA levels did not differ from the saline controls, $F(1,13) = 0.9$, NS.

sparged with nitrogen. The outlet of the swivel joint was connected to the inlet of the probe, and the outlet of the probe led to a 400 μl microcentrifuge vial that was clipped to a flexible cable 4 cm above the head of the rat. Microdialysis probes protruded 5 mm beyond the guide shaft.

For the experiment the probe was inserted, and sample collection started 1.5 hours later. After four consecutive 20-min samples, each of seven rats received an injection of l-sulpiride (20 mg/kg/IP), and seven received saline IP. The l-sulpiride (Revizza) was dissolved in three parts of physiological saline and one part 0.1 N HCl. Saline injections rather than HCl solutions were used as controls as in previous behavioral experiments (1–3). After the injection 10 more samples were collected.

DOPAC, HVA and 5-HIAA were measured by HPLC with

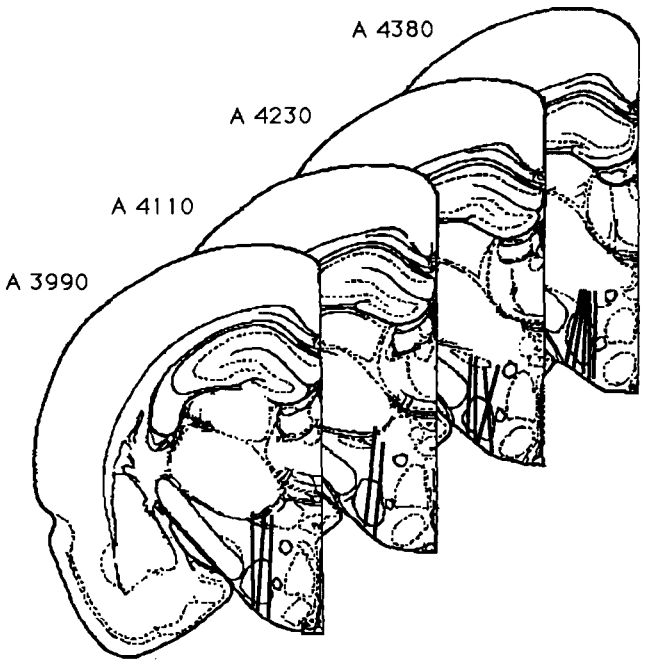

FIG. 2. Black bars show the location of the microdialysis probes in the PFH at planes A3990–A4380 according to the atlas of König and Klippel (13).

electrochemical detection. Separation of the chemicals was carried out by injecting the samples into a Model 7125 Rheodyne valve equipped with a 20 μl loop which fed into a Brownlee 10.0 cm long ODS column, 3.2 mm bore, and 3 μm C-18 packing. A Coulochem 5100A detector (ESA Co.) was used. The buffer was 40 mM phosphate at pH 3.6, with 1.3 mM heptane sulfonic acid (as ion pairing reagent), 238 μM EDTA, and 3% v/v methanol as organic solvent. The Coulochem was set with the guard cell at +500 mV, the conditioning electrode at +100 mV, and the second electrode set in the reduction mode at −350 mV to detect neurochemicals.

At the end of the experiment the rats were sacrificed with pentobarbital; brains were perfused with formalin, frozen and sectioned to localize the probe tracks. For statistical comparison the data were expressed as percent of the first baseline sample, and the sulpiride group was compared to the saline group by a two-way ANOVA for repeated measures followed by the Newman-Keuls test when justified.

Dopamine levels were not detectable in the PFH, and the serotonin levels were not assessed because the buffer and the coulometric parameters were not optimal for serotonin. Concentrations of the chemicals for the 14 rats (pg/20 μl in the first sample, means ± SEM) were as follows: DOPAC 74 ± 8, HVA 850 ± 19 and 5-HIAA 902 ± 97. The preponderance of 5-HIAA may reflect the fact that there is more serotonin than dopamine content in the PFH (20).

After the sulpiride injection DOPAC and HVA increased significantly, but 5-HIAA remained unchanged (Fig. 1). In the saline control group DOPAC decreased about 20% and HVA decreased about 30%. This decrease occurred slowly and might be due to a combination of factors. 1) There is release of metabolites when the probes are inserted due to cell damage. 2) The experiments were carried out in daytime. The rats are awake at the beginning of the experiment and then fall asleep. 5-HIAA served as a control in that a similar decrease was observed both in the sulpiride group and in the saline group. In the case of DOPAC and

HVA, sulpiride not only prevented this monotonic decrease but also increased these metabolites significantly with respect to the first sample.

Figure 2 shows the location of the probes in the lateral hypothalamus according to the atlas of König and Klippel (14).

These results suggest that systemic sulpiride blocked dopamine but not serotonin postsynaptic receptors in the PFH. This dopamine blockade would be expected to cause hyperphagia (15); direct injections of sulpiride in the PFH induced feeding in satiated rats (18), but the direct administration of this drug is not strictly comparable with its systemic administration. We do not have a clear explanation for the sex- and age-dependent effect of sulpiride on body weight in rats; that is, adult and prepubertal female and prepubertal male rats gain body weight, whereas adult males tend to lose weight (2,3). This suggested an endocrine effect that we hypothesized might be related to a decrease in serum gonadal steroid levels (3,19) which increase body weight in female, but not adult male rats (11).

Systemic sulpiride decreases sucrose intake in the sham-feeding paradigm (22). This effect seems to be due to dopamine blockade in the mesolimbic system. Therefore, the same neurotransmitter, dopamine, might have opposite effects on feeding in different brain regions. In fact, amphetamine injections in the nucleus accumbens can increase feeding (6), but amphetamine in the lateral hypothalamus decreases feeding (15,18). It follows that dopamine blockers in the nucleus accumbens might decrease food intake, but in the hypothalamus dopamine blockers might disinhibit feeding.

The results presented here suggest that in the female rat, sulpiride can cross the blood-brain barrier and block the dopaminergic satiety system in the PFH (12, 15, 18). This could increase body weight as seen in both rats (2) and humans (1,7).

ACKNOWLEDGEMENTS

This research was supported by USPHS grant DA-03597, and Universidad de Los Andes grant M-264. Appreciation is expressed to Dawn Davidson and Michelle Fisher for their valuable assistance.

REFERENCES

1. Baptista, T.; Hernandez, L. Efecto de los neurolepticos sobre el peso corporal. XXXI Convencion Anual de ASOVAC; 104; 1981.
2. Baptista, T.; Parada, M.; Hernandez, L. Long-term administration of some antipsychotic drugs increases body weight and feeding in rats. Are D2 dopamine receptors involved? Pharmacol. Biochem. Behav. 27:399–405; 1987.
3. Baptista, T.; Parada, M.; Murzi, E. Puberty modifies sulpiride effects on body weight in rats. Neurosci. Lett. 92:161–164; 1988.
4. Benakis, A.; Rey, C. Sulpiride ^{14}C chez la souris et le rat. Localisation specifique au niveau de l'hypophyse du rat apres administration unique et administrations repetees. J. Pharmacol. 7:367–378; 1976.
5. Carlsson, A. Antipsychotic drugs, neurotransmitters and schizophrenia. Am. J. Psychiatry 135:164–173; 1978.
6. Evans, K. R.; Vaccarino, F. J. Intra-nucleus accumbens amphetamine: Dose-dependent effects on food intake. Pharmacol. Biochem. Behav. 25:1149–1151; 1986.
7. Gopalaswamy, A. K.; Morgan, R. Too many chronic mentally disabled patients are too fat. Acta Psychiatr. Scand. 72:245–248; 1985.
8. Grigoriadis, D.; Seeman, P. The dopamine/neuroleptic receptor. Can. J. Neural Sci. 11:108–113; 1984.
9. Hernandez, L.; Hoebel, B. G. Haloperidol given chronically decreases basal dopamine in the prefrontal cortex more than the striatum or nucleus accumbens as simultaneously measured by microdialysis. Brain Res. Bull. 22:763–769; 1989.
10. Hernandez, L.; Stanley, B. G.; Hoebel, B. G. A small, removable microdialysis probe. Life Sci. 39:2629–2637; 1986.
11. Hervey, E.; Hervey, G. R. The influence of sex hormones on energy balance. In: Cioffi, L. A.; James, W. P. T.; Van Itallie, T. B., eds. The body weight regulatory systems: Normal and disturbed mechanisms. New York: Academic Press; 1981:345–352.
12. Hoebel, B. G.; Hernandez, L.; Schwartz, D. H.; Mark, G. P.; Hunter G. A. Microdialysis studies of brain norepinephrine, serotonin and dopamine release during ingestive behavior: Theoretical and clinical implications. In: Schneider, L. H.; Cooper, S. J.; Halmi, K. A., eds. Psychobiology of human eating disorders. Ann. NY Acad. Sci. 575:171–193; 1989.
13. Imperato, A.; Di Chiara, G. Dopamine release and metabolism in awake rats after systemic neuroleptics as studied by trans-striatal dialysis. J. Neurosci. 5:297–306; 1985.
14. König, J. F. R.; Klippel, R. A. The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brainstem. Baltimore, MD: The Williams & Wilkins Company; 1963.
15. Leibowitz, S. F.; Brown, L. Histochemical and pharmacological analysis of catecholaminergic projections to the perifornical hypothalamus in relation to feeding inhibition. Brain Res. 201:315–345; 1980.
16. Mizuchi, A.; Kitagawa, N.; Miyachi, Y. Regional distribution of sultopride and sulpiride in rat brain measured by radioimmunoassay. Psychopharmacology (Berlin) 81:195–198; 1983.
17. Moore, B. J.; Gerardo-Gettens, T.; Horwitz, B. A.; Stern, J. S. Hyperprolactinemia stimulates food intake in the female rat. Brain Res. Bull. 17:563–569; 1986.
18. Parada, M. A.; Hernandez, L.; Hoebel, B. G. Sulpiride injections in the lateral hypothalamus. Do D_2 receptors mediate dopamine inhibition of feed and drinking? Pharmacol. Biochem Behav. 30:917–923; 1988.
19. Parada, M. A.; Hernandez, L.; Paez, X.; Baptista, T.; Parada, M.; Quijada, M. Mechanism of body weight gain induced by sulpiride. Pharmacol. Biochem. Behav. 33:45–50; 1989.
20. Parada, M. A.; Hernandez, L.; Schwartz, D.; Hoebel, B. G. Hypothalamic infusion of amphetamine increases serotonin, dopamine and norepinephrine. Physiol. Behav. 44:607–610; 1988.
21. Pizzi, M.; Coen, E.; Memo, E.; Missale, C.; Carruba, M. L.; Spano, P. F. Evidence for the presence of D_2, but not D_1 dopamine receptors in rats hypothalamus perifornical area. Neurosci. Lett. 67:159–162; 1986.
22. Smith, G. P.; Schneider, L. H. Relationships between mesolimbic dopamine function and eating behavior. The mesocortical dopamine system. Ann. NY Acad. Sci. 537:254–261; 1988.