INFLUENCES OF HALOPERIDOL AND SULPIRIDE ON SOCIAL BEHAVIOUR OF FEMALE MICE IN INTERACTIONS WITH ANOSMIC MALES

R. REDOLAT, J. MIÑARRO, V. M. SIMON and P. F. BRAIN*

Area de Psicobiología, Facultad de Psicología, Universitat de Valencia.

*Biomedical and Physiological Research Group, Biological Sciences, University College of Swansea, U.K.

ABSTRACT

Most studies on the effects of drugs on social and agonistic behaviour in laboratory animals have involved male subjects. In the present paper the effects of haloperidol and sulpiride on social and defensive behaviour of female mice are assessed using an ethopharmacological method that allows estimation of times allocated to broad behavioural categories. The most remarkable result was that immobility did not experiment any statistically significant increase in animals treated either with haloperidol or sulpiride. Exploratory behaviour was differentially modified by the two drugs: Haloperidol decreased social investigation whereas sulpiride decreased non social explo-ration. Results are related with previous studies (made with male subjects in a similar experimental setup) that suggested the existence of sexual differences in the behavioural effects of these drugs.

Key Words: Haloperidol, Sulpiride, Social interaction, Defensive behaviour female mice.

RESUMEN

In fluencias de Haloperidol y Sulpiride en el comportamiento social de ratón hembra en interacciones con machos anósmicos.- La mayoria de las investigaciones realizadas para estudiar los efectos de los fármacos sobre la conducta social y agonística en animales de laboratorio han utilizado sujetos macho. En este trabajo se evaluan los efectos del haloperidol y el sulpiride sobre la conducta social y defensiva de ratones hembra, utilizando para ello un método etofarmacológico que permite valorar el tiempo que los sujetos dedican a las diferentes categorias conductuales evaluadas. El resultado más destacado fué que la inmovilidad no aumentó significativamente en los animales tratados con haloperidol o sulpiride. La conducta exploratoria resultó afectada de modo diferente por los dos fármacos: El haloperidol disminuyó la investigación social mientras el sulpiride disminuyó la exploración no social. Los resultados se relacionan con estudios previos (realizados con sujetos macho en una situación experimental similar) que sugieren la existencia de posibles diferencias sexuales en los efectos conductuales de estos fármacos.

Palabras Clave: Haloperidol, Sulpiride, Interacción social, conducta defensiva, ratones hembra.

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INTRODUCTION

Haloperidol and sulpiride are clinically proven antipsychotic and sedative agents both being dopaminergic antagonists (Jenner, Clow, Reavill, Theodorov and Mardsen, 1978; Jenner and Marsden, 1979; Kebabian and Calne, 1979). Haloperidol has well-known antiaggressive properties in males (Poshivalov, 1982; Olivier, Van Aken, Jaarsma, Van Oorschot, Zethof, and Bradford,1984; Miñarro, Castaño, Brain, and Simón,1990). Sulpiride also markedly decreases attack behaviour shown by male mice in a social encounter test (Simón, Miñarro, Redolat, and Garmendia, 1989; Redolat, Brain and Simon, -in press-). Most studies on the effects of haloperidol and sulpiride on social and agonistic behaviours have involved male mice. Indeed, there are relatively few studies on females in the general area of behavioural pharmacology (see exceptions in Benton, Smoothy and Brain, 1985 and in Smoothy, Brain, Berry and Haug,1986). It was consequently thought desirable to attempt to provide a detailed analysis of the behavioural actions of haloperidol and sulpiride in female mice.

Resident-intruder tests employing male mice with docile 'standard opponents' have proved useful in studies assessing the anti-aggressive properties of particular compounds. This is so because such situations reliably generate reasonable levels of threat and attack (Brain and Al-Maliki, 1978, Brain, Smoothy and Benton, 1985) with little indication of pronounced defensive or submissive behaviour by the test animals in such situations. Conversely, resident-intruder tests employing non-reproductive female subjects do not generally produce much fighting or threat but they do generate higher base-lines of defensive-submissive and also exploratory behaviour (Benton et al, 1985; Smoothy et al. 1986). For this reason, it was thought that this experimental paradigm might prove appropriate for the assessment of effects of these drugs on defensive behaviour. In the present study, the effects of haloperidol and sulpiride were assessed on social and defensive behaviour by examining the responses of female mice to the presence of a non-familiar anosmic male intruder, in two different experiments.

MATERIALS AND METHODS

Subjects

Ninety-six naive female mice of the Alderley Park strain (from a stock supplied by I.C.I. Pharmaceuticals, Alderley Park, Cheshire, England) were used as experimental subjects (48 for each drug study). They were bred and housed under highly controlled conditions in the Animal Facility of the University College of Swansea. Subjects were weaned at 19-23 days of age and placed in opaque polypropylene cages, measuring 30 x 12 x 11 cm (North Kent Plastics). The subjects were housed in groups of six from weaning until one week before testing. Animals were maintained at 21º C., with a reversed lighting schedule (white lights on 22:30 - 10:30 hrs. G.M.T.), dim red lighting was used at all other times. Food and water were available *ad lib*, except during behavioural trials. An equal number of male mice were housed in groups of six and used as "standard opponents" after being rendered temporally anosmic by intranasal lavage with 4 % zinc sulphate solution, both three days and one day before testing(see Smoothy et al., 1986). Such animals elicit attack by males but do not initiate it. In spite of their lack of threat and attack they stimulate timid behaviour in females (Benton et al., 1985; Smoothy et al., 1986).

Procedure

Drug treatment: Animals were injected i.p. with 0.01, 0.1 or 0.2 mg/kg of haloperidol (Haloperidol®, Latino Laboratories, Spain) or with physiological saline (control group) in a first study. In a second study female mice received acute treatment i.p. with one of the following injections: 31.25 , 62.5 or 125 mg/kg of sulpiride (Dogmatil®, Delagrange, Spain) or physiological saline (control group). Each female was given a single injection.

Social encounters: Twenty or thirty minutes respectively, after haloperidol or sulpiride injection, an anosmic standard opponent (marked with fur dye) was introduced into the female´s cage. The interaction between female and the opponent lasted 10 minutes and was videotaped using a camera sensitive to red light (National Panasonic, model 1350A). During the test, the wire-mesh lid of the cage was replaced with a perforated transparent Perspex cover to facilitate observation. All tests were carried out under dim red illumination between the second and fifth hour of the dark phase of the light/dark cycle on three successive days.

Behavioural Analysis: The tapes were analyzed using a microprocessor (Commodore 64 computer) and a custom-developed program (Brain, McAllister and Walmsley, 1989) which facilitated estimation of times allocated to eleven broad functional behavioural categories. Each category includes a collection of different behavioural postures and elements. The names of categories and their constituent elements are the following: 1) *Body care* (abbreviated groom, self groom, wash, shake, scratch); 2) *Digging* (dig, kick dig, push dig); 3)*Non social exploration* (explore, rear, supported rear, scan); 4) *Explore from a distance* (approach, attend, circle, head orient, stretched attention); 5) *Social Investigation* (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around); 6) *Threat* (aggressive groom, sideways offensive, upright offensive, tail rattle); 7) *Attack* (charge, lunge, attack, chase); 8) *Avoidance/Flee* (evade, flinch, retreat, ricochet, wheel, startle, jump, leave, wall clutch); 9)*Defensive/Submissive* (upright defensive, uprigth submissive, sideways defensive); 10) *Sexual* (attempted mount, mount) and 11) *Immobility* (squat, cringe). A detailed description of all elements can be found in Martinez, Castaño, Simon and Brain (1986) and Martinez, Miñarro, and Simon (1991). This ethopharmacological method allows a complete quantification of the behavioural elements shown by the animal during the social encounters.

Statistical Analysis: Non parametric Kruskal-Wallis tests were performed to assess the variance over different treatment groups. Subsequently two-tailed Mann-Whitney U tests were used to contrast times allocated to each behavioural category by experimental and control groups.

RESULTS

The detailed behavioural results are presented in tables 1 and 2 for haloperidol and sulpiride, respectively.

Kruskal-Wallis analysis showed that there was no significant increase in Immobility in groups treated with different doses of haloperidol and sulpiride i.e. none of the doses used here were ataxic. In spite of this, both drugs significantly changed aspects of exploratory behaviour of female mice during interaction with an anosmic opponent. Kruskal-Wallis revealed significant variance across the categories of Social Investigation in experiment with haloperidol and in Non Social Exploration and Explore-from-a distance in experiment with sulpiride. Halo-

DOSE	saline	0.01mg/kg.	0.1mg/kg.	0.2mg/kg.
BEHAVIOURAL CATEGORY				
(a) **Care of body surface**	4.1	7.8	11	27.1***
	(0-15.2)	(0-35.3)	(0-38.8)	(3.6-172.5)
Digging	5.4	11.5	2.2	19
	(0-84.2)	(0-71.8)	(0-105.72)	(0-84.88)
Non social exploration	278.3	258.8	259.7	301.2
	(163.1-361.8)	(73.9-358.4)	(117.7-378.6)	(183.2-401.8)
Explore a distance	25.4	24.8	60.9	29
	(5.8-98.6)	(9.8-234)	(2.9-199.9)	(11-131)
(b) **Social investigation**	222.8	200.7	117.8 *	135.7 *
	(0-339)	(1.9-301.2)	(16.5-252.8)	(73.9-262.1)
Threat	0	0	0	0
	(0-1)	(0-0.5)	(0-0)	(0-0)
Attack	0	0	0	0
	(0-0.8)	(0-0)	(0-0)	(0-0)
Avoidance/flee	5.3	3.8	5.9	5.3
	(0.3-71)	(1.6-32.4)	(0-23.)	(0-12.91)
Defensive/submissive	11.9	15	69.2	16.9
	(0-259.6)	(0-277.7)	(0-205.6)	(0-154.6)
Sexual	0	0	0	0
	(0-14.9)	(0-14.1)	(0-0)	(0-5.4)
Immobility	0.4	0.3	4.6	0.4
	(0.03-6.8)	(0.03-7.3)	(0.03-60.9)	(0.1-45.1)

(a) Kruskal-Wallis test shows significant variance, $p < 0.015$
(b) Kruskal-Wallis test shows significant variance, $p < 0.024$
*** Differs from control on 2-tailed Mann-Whitney U test, $p < 0.05$.**
***** Differs from control on 2-tailed Mann-Whitney U test, $p < 0.002$**

Table 1. Median times (in seconds) with ranges allocated to broad categories of behaviour from an ethological analysis of social encounters in haloperidol and vehicle treated female mice.

peridol and sulpiride increased explore from a distance (tentative exploration directed by female mice towards the partner during encounter test). This increment was statistically significant with all doses of sulpiride. However, these neuroleptics affect non social exploration (exploration around the cage) and social investigation (directed towards the opponent) in opposite directions. Non social exploration was unchanged by haloperidol but time allocated to this behavioural category significantly diminished with the lowest and intermediate doses of sulpiride. Social investigation decreased in the animals treated with the two higher doses of haloperidol and was increased (although not significantly) in animals treated with sulpiride.

Times allocated to threat and attack were, as expected, minimal in all categories used here and there were no statistical differences between animals treated with sulpiride or haloperidol and their control groups. Neither haloperidol nor sulpiride significantly reduced defensive or submissive behaviours.

Haloperidol and sulpiride increased the time devoted to body care, although this increase only was statistically significant for the higher dose of haloperidol. Neither haloperidol nor sulpiride changed significantly digging behaviour.

DISCUSSION

Ethopharmacological assessment of the behaviour of female mice when confronted with 'standard opponents' showed

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DOSE	saline	31.25 mg/kg	62.5 mg/kg	125 mg/kg
BEHAVIOURAL CATEGORY				
Care of body surface	14.3 (1.5-53.6)	24.1 (68-52.6)	25.5 (3.8-88.7)	35.4 (0-86.7)
Digging	13.4 (0-69.5)	13 (0-76.3)	3.4 (0-22.6)	20.2 (0-49.5)
(a) Non social exploration	380.2 (306.6-481.2)	306.6** (208.6-387.3)	283.4** (222.8-404.7)	335.6 (258.7-470.5)
(b) Explore a distance	14.6 (5.4-33)	30.5** (7.2-37)	52.1*** (17.6-88.5)	40.8* (10-51.4)
Social investigation	57.7 (0-204.6)	121.3 (2.9-186.8)	107.5 (2.9-254.2)	63.7 (0-151.1)
Threat	0 (0-5.5)	0 (0-13.9)	0 (0-3.1)	0 (0-4.1)
Attack	0 (0-0)	0 (0-4.5)	0 (0-10.8)	0 (0-1.2)
Avoidance/flee	45.4 (4.8-108.9)	37.8 (2.2-97.2)	32.6* (.7-85.6)	26.5 (1.7-92.7)
Defensive/submissive	20.9 (0-128.8)	23.4 (0-248)	32.9 (0-111.2)	20.2 (0-210)
Sexual	0 (0-0)	0 (0-1.8)	0 (0-10)	0 (0-3.8)
Immobility	.175 (.03-3)	.32 (.02-5.5)	.5 (.02-13.5)	.6 (.02-14.9)

(a) Kruskal-Wallis test shows significant variance, $p < 0.006$.
(b) Kruskal-Wallis test shows significant variance, $p < 0.001$.
*** Differs from controls on 2-tailed Mann-Whitney U test, $p < 0.05$**
**** Differs from controls on 2-tailed Mann-Whitney U test, $p < 0.02$**
***** Differs from controls on 2-tailed Mann-Whitney U test, $p < 0.002$**

Table 2. Median times (in seconds) with ranges allocated to broad categories of behaviour from an ethological analysis of social encounters in sulpiride and vehicle treated female mice.

that exploratory behaviour were specifically changed, although differently by the two drugs: sulpiride diminished non-social exploration and increased explore-from-a-distance and social investigation. These results may reflect an increased "interest" by female mice towards the males. Because of this increase in time spent exploring the opponent, time devoted to non social exploration was reduced. Conversely, haloperidol significantly decreased time devoted to social investigation but did not significantly change time allocated to non-social exploration by female mice. This differential effect could be attributed to the well known motor effects of haloperidol. One could argue that social investigation is an activity that requires more energy and coordination than non social exploration. Consequently, social investigation (interest towards the opponent) would be more sensitive to sedative actions of haloperidol. Effects of these drugs on social exploratory behaviour are not specific for female mice. In previous studies we found that haloperidol diminishes social investigation in males whereas sulpiride increases this behaviour (Miñarro et al., 1990; Redolat et al. -in press-).

This differential effect of haloperidol and sulpiride on exploratory behaviour could be related with the selective effects of these drugs in neural dopaminergic system. Both drugs are dopaminergic antagonists, having more afinity for the D2 than for the D1 receptors (Hyttel and Arnt, 1986), although they diferentially alter dopamine turnover. Experimental measurements of regional levels of Homovanillic acid (HVA),

the main metabolite of Dopamine, after acute administration of haloperidol and sulpiride showed that haloperidol induces larger concentrations of HVA in the striatum than in the limbic areas (Bartholini, 1976) whereas sulpiride increases dopamine turnover more in the limbic system than in the striatum (Bartholini and Lloyd, 1980). These differences could explain the fact that haloperidol has greater effect than sulpiride on activities with greater motor components (i.e. social investigation)

In agreement with previous studies (Benton et al., 1985; Smoothy et al., 1986), moderate levels of "avoidance-flee" and "defensive-submissive" behaviour and an absence of aggressive postures were evident although the males were anosmic and did not attack. Some female mice very frequently showed defensive postures when males approached them. Defensive behaviour was uninfluenced by any of the administered doses of haloperidol and sulpiride.

In the present study, haloperidol increased significantly body care while previous data had shown that this parameter significantly diminished in male mice treated with haloperidol (Miñarro et al., 1989).

One of the most remarkable results was that neither haloperidol nor sulpiride significantly increased immobility. This fact is specially salient wiht regard to haloperidol. Previous data showed that haloperidol strongly increased immobility in male mice. This has been observed when male mice are confronted with standard opponents in a neutral arena (Miñarro et al., 1990) or in their own cage (Miñarro, Brain and Simon,1989).

Comparing present results with previous studies (Miñarro et al., 1990; Redolat et al., -in press-) obvious differences in social behaviour appear between males and females. These results support previous data about sex differences in the behavioural effects of neuroleptic drugs in animals (Dalton, Vickers and Roberts, 1986) and man (Swet, 1975).

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Address correspondence to: Dr. V. M. Simon
Area de Psicobiología, Facultad de Psicología
Universitat de Valencia, Apartado 22109,
46071 Valencia, SPAIN