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TIME COURSE OF THE EFFECTS OF HALOPERIDOL ON AGONISTIC BEHAVIOUR IN MALE MICE

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The effects of haloperidol on aggressive behaviour 24 hours after administration have been studied previously with contradictory results. This study is an attempt to determine the persistence of the antiaggressive and motor effects of 0.4 mg/kg of haloperidol over time, studying its actions on agonistic behavior in male mice at 30 minutes, at 24 and at 48 hours after administration. When the test is performed 30 minutes after administration, there is a significant decrease of time allocated to offensive behaviors and nonsocial exploration and a significant increase in time devoted to immobility behavior in the animals that received haloperidol with respect to their controls. No significant effects were found 24 or 48 hours after administration. Differences with similar studies are commented upon.

Key words: Haloperidol, Aggression, Agonistic Behavior, Mice, Time-course.

Curso temporal de los efectos del Haloperidol sobre la conducta agonística en ratones macho. Los efectos del haloperidol sobre la conducta agresiva a las 24 horas de su administración han sido estudiados previamente con resultados contradictorios. En este trabajo se intentó determinar la persistencia a través del tiempo de los efectos antiagresivos y motores de 0.4 mg/kg de haloperidol sobre la conducta agonística de ratones macho, estudiando sus acciones a los 30 minutos, a las 24 y a las 48 horas de la administración. Cuando el test fue realizado a los 30 minutos de la administración, se encontró una disminución del tiempo dedicado a las conductas ofensivas y a la exploración no social y un aumento del tiempo de inmovilidad en los animales que recibieron haloperidol con respecto a sus controles. A las 24 ó 48 horas de la administración no se encontraron diferencias significativas. Se comentan las diferencias encontradas con estudios similares.

Palabras clave: Haloperidol, Agresión, Conducta agonística, Ratones, Curso temporal.

In a previous study (Navarro, Miñarro and Simón, 1993) it was observed that 24 hours after administration of haloperidol, this drug still showed antiaggressive effects while immobility effects disappeared. Likewise, other studies have shown prolonged behavioural effects after one moderate dose of the drug (Cohen, Babb, Campbell and Baldessarini, 1988). Typically quoted half-lives for neuroleptics, including haloperidol, are approximately 24 hours or less (Baldessarini, 1990); however, there is suggestive evidence that elimination of haloperidol slows with time after dosing (Hubbard, Ganes and Midha, 1987) and that the near terminal elimination half-life may be measured in days rather than hours (Cohen et al, 1988). Recvently, Cohen, Tsuneizumi, Baldessarini, Campbell and Babb (1992) have determined the persistence of haloperidol (1 mg/kg ip) in rat plasma and brain tissue, using high pressure liquid cromatography. In this study, the authors found that plasma le-

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vels of the drug were not detectable at times longer than 2 days after injection; however, the half-live of haloperidol in the brain was many times greater (ca. 6-7 days).

Zetler and Bauman (1985) have studied the pharmacokinetics of haloperidol in the mouse serum and brain. After subcutaneous administration of 0.6 mg/kg, the absorption of the drug was very fast so that after 2 minutes it had reached already its maximal serum concentration which began to decrease only after 15 minutes. In the mouse brain the concentration of haloperidol rises during the first 30 minutes up to a 7-fold of its serum level. After these times, the drug levels in serum and brain decreased with a phase of rapid decline in the first 6 hours and a second slower phase of decline (12 hours approximately in serum, and between 12 and 24 hours in the brain). Thus, brain levels were always considerably higher than those in serum (Zetler and Baumann, 1985).

Haloperidol, like other neuroleptics, shows obvious antiaggressive effects. Most of the relevant studies have focussed on its acute effects (with the range of doses between 0.1 and 2.5 mg/kg) and a significant reduction in aggressive behaviour has been described using different animal models: predatory aggression (Delini-Stula and Vassout, 1979), clonidine induced aggression (Fujiwara, Takeda, Kazahaya, Otsuki and Sandyk, 1988), electric-shock induced aggression (Nakao, Higashio and Inukai, 1985), hypothalamic stimulation induced aggression (Olivier, van Dalen and Hartog, 1986), maternal aggression (Yoshimura and Ogawa, 1989) and isolation-induced aggression (Olivier et al, 1986; Miñarro, Castaño, Brain and Simón, 1990; Navarro, Miñarro and Simón, 1992). This decrease in aggression is generally accompanied by a concomitant reduction of motor behaviour being both effects difficult to separate. However, studies using atypical neuroleptics, like sulpiride, clozapine, or raclopride, have shown a reduction of aggressive behaviour without significant disruption of motility (Redolat, Brain and Simón, 1991; Garmendia, Sánchez, Azpiroz, Brain and Simón, 1991; Aguilar, Miñarro, Perez-Iranzo and Simón, 1994). It has also been observed that after the acute or chronic (15 or 30 days) administration of haloperidol the time courses of the antiaggressive and motor effects are divergent. These findings support the hypothesis that the antiaggressive effects of neuroleptics are produced independently of the motor ones and suggest that the neurophysiological processes underlying aggression and motility are different (Navarro et al., 1993).

This study is an attempt to determine the persistence of the antiaggressive and motor effects of haloperidol (0.4 mg/kg, i.p.) over time, studying its actions on agonistic behaviour in male mice, at 30 minutes, 24 and 48 hours after administration.

MATERIAL AND METHODS

Subjects

144 male albino mice of the OF 1 strain, acquired in Iffa Credo (Barcelona, Spain) were used. The animals arrived at the laboratory aged 42 days, and were housed under standard laboratory conditions; constant temperature (21° C), a reversed light schedule (white lights on: 0100-1300 h.) with food and water available ad lib except during behavioural testing. 72 mice were housed individually in transparent plastic cages (24 × 13.5 × 13 cm) and were employed as experimental and control animals. The remaining 72 were housed in groups of 6 in bigger cages (25 × 25 cm × 13.5) to be used as standard opponents and were made temporally anosmic by intranasal lavage with a 4% zinc sulphate solution on both 1 and 3 days before testing. These animals elicit the attack of the experimental males but dont initiate it, since they cannot perceive a pheromone present in the urine of the experimental animals, which is considered to be a cue for eliciting

aggressive behaviour in mice with a normal sense of smell (Mugford and Nowell, 1970).

Procedure

Drug treatment: Animals were injected IP only once with 0.4 mg/kg haloperidol (Haloperidol ®, Latino Laboratories, Spain) or physiological saline (ClNa at 0.9%).

Social encounters. Tests were performed at 30 minutes, and at 24 or 48 hours after drug administration. An experimental animal and an anosmic opponent were introduced in a neutral glass cage (60 x 30 x 30 cm) and their interaction (which lasted 10 minutes) was videotaped for later behavioural analysis. Tests were performed between the second and eighth hour of the dark phase of the light/dark cycle. For each experimental animal a different anosmic opponent was used.

Behavioural analysis: The tapes were analyzed using a microprocessor (Commodore 64 computer) and a custom-developed program that facilitated estimation of times allocated to eleven broad functional behavioural categories (Brain, McAllister and Walmsley, 1989). Each category included a collection of different behavioural postures and elements: 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); and 8) Immobility (squat, cringe). A detailed description of all elements can be found in Martínez, Miñarro, and Simón (1991). The analysis of the videotape involved assessment only of the behaviour of the experimental animals. This analysis was performed by a trained experimenter who was «blind» as to the experimental group to which each animal belonged.

Statistical analysis: The medians for times allocated to each broad behavioural category were determined, and appropiate paired comparisons were carried out using Mann-Whitney U tests to contrast the behaviour in different treatment groups.

RESULTS

Table 1 illustrates medians (with ranges) of accumulated times allocated to the broad categories of behavior described above, showing the significant differences between each group and its control (*) and the significant differences between the three groups treated with haloperidol (Δ).

In tests performed 30 minutes after haloperidol administration, treated animals showed, with respect to controls, a significant decrease ($p<0.002$) of time allocated to offensive behaviours (threat and attack) and non social exploration behaviour ($p<0.02$), and a significant increase in time devoted to immobility ($p<0.002$).

A similar statistical analysis carried out in the groups that performed the test 24 and 48 hours after drug administration showed no significant differences with respect to their controls in any of the behavioural categories.

Among the three control groups no significant differences were found, making it possible to compare the three haloperidol treated groups. Animals spent significantly more time in attack and non social exploration behaviours in the groups that performed the test at 24 hours ($p<0.02$) and at 48 hours ($p<0.002$) with respect to the group that performed it at 30 minutes. Moreover the group tested 48 hours after drug administration spent significantly more time in threat behaviour than the one tested at 30 minutes ($p<0.05$). It was also observed that there was a decrease in the time devoted to immobility in the groups of 24 and 48 hours with respect to the group of 30 minutes ($p<0.002$).

Table 1
Median values (with ranges) for times (in seconds) allocated to broad behavioural categories

BEHAVIOURAL CATEGORIES	TIME INTERVAL BETWEEN INJECTION AND TEST					
	30 minutes		24 hours		48 hours	
	Saline	Haloperidol	Saline	Haloperidol	Saline	Haloperidol
Body care	13 (1-32)	8 (0-20)	11 (1-16)	11 (0-95)	11 (0-35)	15 (2-43)
Digging	12 (0-38)	5 (0-34)	14 (0-27)	10 (0-30)	8 (0-33)	20 (0-40)
Non social exploration	281 (229-375)	187** (62-322)	304 (215-423)	321 ΔΔ (171-390)	295 (166-406)	318 ΔΔΔ (249-408)
Explore from a distance	38 (19-79)	34 (16-109)	33 (17-71)	33 (15-66)	33 (19-59)	37 (17-90)
Social Investigation	59 (13-138)	71 (6-113)	103 (35-261)	206 (13-233)	76 (21-206)	55 (11-274)
Threat	102 (34-175)	25*** (0-119)	79 (0-114)	73 (0-240)	88 (0-145)	52 Δ (0-95)
Attack	66 (13-135)	0*** (0-18)	46 (0-114)	37 ΔΔ (0-86)	59 (0-260)	52 ΔΔΔ (0-93)
Immobility	0 (0-13)	227*** (113-453)	0 (0-7)	0 ΔΔΔ (0-1)	0 (0-1)	0 ΔΔΔ (0-40)

** differs from its control on two-tailed Mann-Whitney U test, $p<0.02$.
*** differs from its control on two-tailed Mann-Whitney U test, $p<0.002$.
Δ differs from «Haloperidol 30 min.» on two-tailed Mann-Whitney U test, $p<0.05$.
ΔΔ differs from «Haloperidol 30 min.» on two-tailed Mann-Whitney U test, $p<0.02$.
ΔΔΔ differs from «Haloperidol 30 min.» on two-tailed Mann-Whitney U test, $p<0.002$.

No significant differences between the groups that performed the test at 24 hours and 48 hours after haloperidol administration were found.

DISCUSSION

When the tests were performed 30 minutes after haloperidol administration the results confirmed the findings of previous studies (Delini-Stula and Vassout, 1979; Miñarro et al, 1990). Haloperidol showed an antiaggressive effect diminishing threat and attack behaviours and on the other hand it had obvious effects on motor behaviour, decreasing nonsocial exploration and increasing immobility.

In this study no significant differences between the group which performed the test 24 hours after haloperidol administration and its control group were found, showing that at this time the antiaggressive and motor effects of the drug had disappeared (only a tendency for attack behaviour to decrease and for nonsocial exploration and social investigation to increase were present). On the contrary, previous studies on the effects of haloperidol on aggressive behavior 24 hours after administration had obtained different results. Navarro et al.

(1993) investigated the effect of acute administration of 0.4 mg/kg of haloperidol 24 hours after administration and found significant decreases in threat and attack with respect to controls. However, Puigcerver et al. (-in preparation) in a similar study carried out 24 hours after drug administration only found a significant decrease in nonsocial exploration. The present study and those previously discussed have used animals of the same strain of mice and employed the same methodology, although they were carried out in different laboratories and by different experimenters.

In the group which performed the test 48 hours after drug administration no significant effects of haloperidol were found in any behavioral categories, as one would expect. No similar studies concerning aggression have been found in the literature. In other behaviours, such as apomorphine-induced stereotypies, the effects of haloperidol have been found even 21 days after administration. It is, nevertheless, interesting to point out that this anomalous behavior appears to be more sensitive than spontaneous behaviors like aggression, for detecting long-lasting effects of neuroleptics (Cohen et al., 1992).

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