José Eduardo Tanus dos Santos

ALTERAÇÕES INDUZIDAS PELA DESNUTRIÇÃO PÓS-NATAL NOS EFEITOS COMPORTAMENTAIS DE ANTAGONISTAS DOPAMINÉRGICOS EM RATOS JOVENS

Dissertação de Mestrado apresentada ao Curso de Pós-Graduação em Farmacologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do título de Mestre em Ciências na área de Farmacologia.

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SUMÁRIO

A desnutrição durante períodos críticos do desenvolvimento pode induzir alterações nas respostas comportamentais às drogas de ação central. Neste estudo, avaliaram-se os efeitos da desnutrição pós-natal sobre as respostas comportamentais de ratos com 21 dias de idade a antagonistas dopaminérgicos. Utilizou-se clorpromazina (0, 2,5, 5, 10 and 20 mg/kg), haloperidol (0, 0,125, 0,25, 0,5, 1 or 2 mg/kg) e SCH23390 (0, 0,3, 0,6 and 1,2mg/kg). Avaliou-se a atividade locomotora 1h 30min, 4h 30min, 7h 30min e 10h 30min após a injeção de clorpromazina ou haloperidol. A catalepsia induzida por estas drogas foi avaliada 3h, 6h e 9h após a injeção. Os efeitos SCH23390 foram avaliados 25 min, 1h 10 min, 1h 55min e 2h 40min após a injeção. Todas as drogas foram injetadas em dois dias consecutivos. No primeiro dia, os ratos desnutridos tratados com salina apresentaram atividade locomotora mais intensa do que os normais. A inibição da atividade locomotora foi menos intensa nos ratos desnutridos do que nos animais normais 4h 30min após a injeção de clorpromazina e 7h 30min após a injeção de haloperidol. O SCH23390 produziu efeitos similares sobre a atividade locomotora dos animais normais e desnutridos. No segundo dia houve uma tendência a respostas menos intensas dos animais desnutridos em comparação com os normais, apenas para os animais que receberam 5 mg/kg de clorpromazina ou 0,5 , 1 e 2 mg/kg de haloperidol. No primeiro dia, os animais desnutridos tiveram respostas cataleptogênicas menos intensas à clorpromazina e ao haloperidol 6h após a injeção. Os efeitos cataleptogênicos do SCH23390 foram menos intensos nos animais desnutridos 1h 55min após a injeção. No segundo dia, os efeitos das três drogas sobre a catalepsia foram menos intensos nos animais desnutridos, porém as diferenças não foram tão evidentes quanto aquelas notadas no primeiro dia. Estes resutados sugerem que os efeitos comportamentais da clorpromazina, haloperidol e SCH23390 sejam menos persistentes nos ratos desnutridos quando comparados com os efeitos em animais normais. Estas diferenças podem ser devidas a alterações farmacocinéticas induzidas pela desnutrição.

INTRODUÇÃO

O sistema nervoso central (SNC) das várias espécies de mamíferos apresenta um desenvolvimento extremamente acentuado durante o período perinatal, observando-se alterações morfológicas, fisiológicas, bioquímicas e comportamentais. Vários sistemas enzimáticos envolvidos no metabolismo de neurotransmissores e neuromoduladores, bem como o número de receptores e sítios de recaptação sofrem alterações neste período. Como conseqüência deste intenso desenvolvimento, o sistema nervoso torna-se muito vulnerável a fatores ambientais durante este período. Entre eles, o fator nutricional tem sido muito estudado. Já foi demonstrado que a desnutrição durante os períodos iniciais do desenvolvimento pode causar alterações neuroquímicas e comportamentais permanentes em animais (Crnic 1983; Marichich et al.,1979; Morgane et al., 1978; Resnick et al.,1979; Rocha & Venedite, 1990; Smart et al., 1989; Tonkiss et al., 1987; Wiggins et al., 1984). Vários estudos revelaram que a desnutrição no período perinatal causa mudanças transitórias ou permanentes nas atividades de enzimas envolvidas em vários sistemas de neurotransmissores (Morgane et al., 1978; Represa et al., 1989; Rocha et al., 1990; Rocha & Souza, 1994; Wiggins et al., 1984) bem como nas concentrações teciduais de várias classes de neurotransmissores (Marichich et al., 1979; Morgane et al., 1978; Wiggins et al., 1984; Venedite et al., 1988). Contudo, nem sempre se obteve uma relação direta entre uma determinada atividade enzimática e o neurotransmissor a ela relacionado (Wiggins et al. 1984).

Estas alterações nos estados funcionais de vários sistemas de neurotransmissores suscitou investigações no sentido de se esclarecer se a desnutrição determinaria modificações nas respostas comportamentais às drogas psicoativas, o que foi feito utilizando-se drogas atuantes em diferentes sistemas de neurotransmissores. Assim, baseados no fato de que a desnutrição protéica muda as concentrações cerebrais de triptofano e serotonina (Resnick & Morgane, 1984), Hall et al. (1983) investigaram os efeitos da desnutrição sobre as respostas comportamentais de ratos adultos ao agonista serotoninérgico N,N-metiltriptamina. Eles observaram que os

animais desnutridos eram menos sensíveis à droga em quatro diferentes tipos de comportamento. Outros estudos demonstraram que a desnutrição nas fases iniciais do desenvolvimento diminui as concentrações de β-endorfinas hipotalâmicas em ratos adultos, além de diminuir as respostas à administração de opióides exógenos e ao naltrexone em várias situações comportamentais (Perry et al., 1990; Rotta et al., 1988; Rocha & Mello, 1994; Souza et al. 1992; Venedite et al., 1987;1988). Vários outros estudos demonstraram que animais submetidos à desnutrição proteica precoce apresentam, quando adultos, alterações nas respostas às drogas de ação central (Brioni & Orsingher 1988; Cordoba et al., 1990; Leahy et al. 1978). Por outro lado, apenas alguns estudos investigaram o efeito destas drogas em ratos jovens (Goodlett et al., 1985; Souza et al., 1992).

O estudo dos efeitos de drogas de ação central realizado com ratos, especificamente durante a fase de crescimento inicial, é de muito interresse, pois ratos desnutridos podem apresentar alterações nas concentrações de noradrenalina, serotonina e dopamina no SNC (Morgane et al., 1978; Resnick & Morgane, 1984; Wiggins et al; 1982;1984). Ainda, o sistema dopaminérgico e vários outros sistemas de neurotransmissores se desenvolvem intensamente durante o período pós-natal precoce, o que pode ser evidenciado tanto por métodos bioquímicos (Broaddus & Bennett Jr 1990; Muller et al., 1994; Pardo et al., 1977) quanto por estudos de psicofarmacologia (Baez et al., 1976; Coyle et al., 1985; Shalaby & Spear 1980; Smith et al., 1982). Estes fatos evidenciam a grande susceptibilidade aos distúrbios induzidos pela desnutrição durante o início do desenvolvimento do SNC.

O presente trabalho objetiva avaliar os efeitos comportamentais de diferentes antagonistas dopaminérgicos, a saber: clorpromazina, haloperidol e SCH23390, em ratos jovens submetidos à desnutrição pós-natal. Estas drogas possuem diferentes estruturas químicas e diferentes ações sobre receptores dopaminérgicos do tipo D1 e D2 (Tamminga & Gerlach 1987) e foram escolhidas como "ferramentas" de estudo do sistema dopaminérgico, devido ao fato de alguns autores terem demonstrado, previamente, que ratos

desnutridos apresentam menor número de sítios de ligação para o [^{3} H]-espiroperidol em membranas de estriado e hipossensibilidade à apomorfina (agonista dopaminérgico), bem como maiores concentrações de dopamina em tecidos nervosos centrais (Wiggins et al. 1982; 1984). Portanto, o presente estudo objetivou investigar se o fenômeno de hipossensibilidade dos ratos desnutridos, previamente descrito em relação aos agonistas dopaminérgicos, também se verificaria quando da administração de antagonistas dopaminérgicos. Ainda, decidimos avaliar, comparativamente, os efeitos de cada uma destas drogas, decorridos vários intervalos de tempo após a injeção, tanto em animais desnutridos como em animais normais. Isto merece atenção especial, pois a maioria dos estudos prévios avaliaram efeitos de drogas em apenas um momento após sua administração. Ainda, na maioria dos estudos que procuraram avaliar as influências da desnutrição, utilizaram-se dietas pobres em proteínas (Almeida et al. 1996; Goodlett et al. 1985; Hall et al. 1983; Leahy et al. 1978; Perry et al. 1990; Rocha & Mello 1994; Venedite et al 1987). Neste estudo, a desnutrição foi realizada reduzindo-se a quantidade de alimento ofertada às fêmeas no período pós-natal, quando estas amamentavam seus filhotes. Avaliamos a inibição da atividade locomotora e a catalepsia induzidas por estes antagonistas dopaminérgicos. Estas duas formas de avaliação do comportamento parecem ser controladas por diferentes vias neurais, ambas dopaminérgicas (Beninger 1983; Campbell et al. 1988; Fink & Smith 1980; Museo & Wise 1990). A catalepsia parece estar relacionada aos efeitos extrapiramidais dos antagonistas dopaminérgicos (Campbell et al. 1988), ao passo que os efeitos sobre a atividade locomotora parecem estar mais relacionados ao antagonismo de receptores dopaminérgicos de outras localizações, tais como os receptores do sistema mesocorticolímbico (Fink & Smith 1980; Museo & Wise 1990).

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Dear colleague,

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MS 6507 - Undernutrition During Sickling Changes the Sensitivity to Haloperidol and Chlorpromazine in Two Behavioural Measures in Weaning Rats

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Undernutrition During Suckling Changes the Sensitivity to Haloperidol and Chlorpromazine in Two Behavioral Measures in Weaning Rats

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Running Title: Undernutrition and sensitivity to chlorpromazine and haloperidol

Abstract: Undernutrition during critical periods of development may cause changes in the behavioral responses of rats to centrally acting drugs. In the present study, the effects of undernutrition during suckling on the behavioral responses of 21-days-old rats to chlorpromazine (0, 2.5, 5, 10 and 20 mg/kg) or haloperidol (0, 0.125, 0.25, 0.5, 1 or 2 mg/kg) were examined. Locomotion was assessed at 1h30min, 4h30min, 7h30min and 10h30min, and catalepsy was scored at 3h, 6h and 9h after drug administration. Drug was injected on two consecutive days. On day 1, saline-treated undernourished rats showed significant greater locomotion activity than did normal rats. The neuroleptic-induced inhibition of locomotor activity in undernourished rats was significantly less than that observed in normal rats from 4h30min to 10h30min (chlorpromazine) or from 7h30min to 10h30min (haloperidol). On day 2, a similar trend was observed but only in rats injected with 5 mg/kg chlorpromazine or 0.5, 1, and 2 mg/kg haloperidol. On day 1, the catalepsy scores at 3 h revealed no significant difference between nutritional groups, but at 6h undernourished rats responded significantly less to chlorpromazine or haloperidol. On day 2, undernourished rats were less responsive to neuroleptics than normal rats, but the effect was not so evident as observed on day 1. The present results suggest that the behavioral effects of chlorpromazine and haloperidol are less persistent in undernourished rats, possibly due to differences in drug distribution and elimination, when compared to well-nourished rats.

Undernutrition during early periods of development may cause permanent alterations in the neurochemistry and behavior of experimental animals (Crnic 1983; Marichich et al. 1979; Morgane et al. 1978; Resnick et al. 1979; Rocha & Vendite, 1990; Smart et al. 1989; Tonkiss et al. 1987; Wiggins et al. 1984). Various studies have demonstrated that perinatal undernutrition causes transitory or permanent changes in enzyme activities related to various neurotransmitter systems (Morgane et al. 1978; Represa et al. 1989; Rocha et al. 1990; 1991; Rocha & Souza, 1994, Wiggins et al. 1984) as well as changes in the levels of various classes of neurotransmitters (Marichich et al. 1979; Morgane et al. 1978; Wiggins et al. 1984; Vendite et al. 1988).

Changes in the functional state of neurotransmitter systems prompted investigators to examine whether they modify the behavioral sensitivity of undernourished rats to psychoactive drugs. On the basis of the fact that protein malnutrition changes the cerebral levels of thryptophan and serotonin (Resnick & Morgane, 1984), Hall et al.(1983) investigated the effects of malnutrition on the behavioral response of adult rats to the serotonergic agonist, N,N-methylthryptamine. They observed that malnourished animals were less sensitive to the drug in four behavioral tasks. Other studies have shown that early undernutrition decreases the level of hypothalamic ß-endorphin in adult rats and causes hyposensitivity to exogenously administered opioid peptides and to naltrexone in various behavioral situations (Perry et al. 1990; Rotta et al. 1988; Rocha & Mello, 1994; Souza et.al. 1992; Vendite et al. 1987; 1988). Several other studies have demonstrated that animals undernourished during pregnancy and/or suckling present alterations in response to drugs acting in the central nevous system during adulthood (Almeida et al. 1996; Brioni and Orsingher 1988; Cordoba et al. 1990; Leahy et al. 1978). In contrast, only a limited number of studies have investigated the effect of drugs in young rats (Goodlett et al. 1985; Souza et al. 1992).

Investigation of drugs that exert their effects centrally in developing rats is of considerable interest, since undernourished young rats may present

changes in the levels of nor-epinephrine, serotonin and dopamine. (Morgane et al. 1978; Resnick & Morgane, 1984; Wiggins et al. 1982; Wiggins et al. 1984). Furthermore, dopaminergic and various other neurotransmitter systems display intense development during the early postnatal period which can be evidenced by biochemical (Broaddus & Bennett Jr 1990; Muller et al. 1994, Pardo et al. 1977) or psychopharmacological approaches (Baez et al. 1976; Coley et al. 1985; Shalaby & Spear 1980; Smith et al. 1982). Consequently, during early development the potential susceptibility to nutritional insult increases.

The current study evaluates the behavioral effects of two antipsychotic agents (chlorpromazine and haloperidol) with different chemical structures and with distinct anti-dopaminergic action (Tamminga & Gerlach 1987) on weaning undernourished rats. The rational for the use of these drugs resides in the fact that previous studies have indicated that weaning undernourished rats present a low density of spiroperidol binding sites and elevated levels of dopamine (Wiggins et al. 1982; 1984). Furthermore, the effects of chlorpromazine and haloperidol were assessed at various intervals in order to determine whether undernourished and normal rats showed similar behavioral responses to drug as a function of time. This aspect deserves attention because the majority of the previous studies have evaluated the effects of drugs in undernourished animals using a single time point. Another important aspect is that these previous studies that evaluated the effects of drugs in undernourished animal have used a low protein diet to undernourish dams and pups (Almeida et al. 1996; Goodlett et al. 1985; Hall et al. 1983; Leahy et al. 1978; Perry et al. 1990; Rocha & Mello 1994; Vendite et al. 1987). In the present study, undernutrition was induced by reducing the quantity of food offered to dams during suckling. Different methods used to induce undernutrition change maternal behavior and other non-nutritional variables in distinct ways (Crnic 1980; Fleischer & Turkewitz 1981; Frankova 1973; Galler & Kanis, 1987; Galler & Turkewitz 1977; Lynch 1976; Rocha et al. 1988; Rocha & Vendite

1990; Smart 1983; Wiener et al. 1977), which may influence the behavioral response to drugs. Catalepsy and locomotion, two classes of behavior that are presumably controlled by different pathways of the dopaminergic system (Beninger 1983; Campbell et al. 1988; Fink and Smith 1980; Museo and Wise 1990) were investigated. Catalepsy seems to be related to extrapyramidal effects of neuroleptics (Campbell et al. 1988) and locomotion seems to be associated with pathways located in other brain regions such as the mesocorticolimbic dopamine system (Fink and Smith 1980; Museo & Wise 1990).

Material and Methods

Undernutrition. Wistar rats from our own breeding stock were used. Rats were maintained on a 12h light/12 h dark cycle (light on at 700).Three virgin female rats were housed with one male for about 20 days. After this period, pregnant rats were individually caged in opaque plastic cages (48 x 22 x 18 cm). On the day of birth (day 0), litters were adjusted to 9 pups and half the dams were assigned at random to one of the nutritional groups. Normal dams had free access to a commercial diet, while undernourished mothers received a restricted food regimen. On day 0, food was removed and thereafter the following food scheme was applied to the undernourished dams: from day 1 to 7 they received approximately 12 g of food/day, from day 8 to 14 approximately 17 g, and from day 15 to 22 approximately 25 g/day. Water was available at all times to rats of both nutritional treatments. The food restriction imposed resulted in a decrease in the amount of milk avaliable to the pups. This method is similar to those used by various investigators (Smart & Dobbing 1971; Crnic & Chase 1978; Stephens & Tonkiss 1980; Rogers et al. 1986). However, the nutritional deprivation was more severe since the restricted food offered to undernourished dams amounted only to about 40% of the intake of normal dams and resulted in the loss of about 20% of the litters (Rocha et al. 1993).

Chlorpromazine treatment. On day 20, female offspring of both dietary groups were weighed and randomly assigned to one of the following doses of chlorpromazine: 0 (saline treated group), 2.5, 5, 10 or 20 mg/kg. On day 21, rats were weighed and injected subcutaneously with chlorpromazine (10 ml/kg of body weight). Twenty four hours after the first drug administration (day 22), rats were weighed and injected with the same dose used on day 1. Rats within a litter were assigned at random to a given dose but in such a way that no more than one rat from a single litter was assigned to the same dose. Rats were returned to their home cage immediately after each injection.

Haloperidol treatment Rats were treated in a similar way to that as described for chlorpromazine, except that the following doses were used: 0 (saline treated rats), 0.125, 0.25, 0.50, 1 and 2 mg/kg.

Open field behavior. Locomotion was assessed by the number of squares crossed with the four paws and rearing responses by the number of times the rats stood up on their hind legs in an open-field arena measuring 56 x 42 x 40 cm (high) which had the floor divided into 12 squares (Pereira et al. 1992). Each open-field session lasted 3 min and was carried out 1hr 30 min, 4 hr 30 min, 7 hr 30 min and 10 hr 30 min after each daily injection. The open field apparatus was located in a fixed place on the floor of the colony room. Each rat was removed from the home cage just before testing (the place of the home cages in the colony room was not changed during the experiments) and returned to it just after the end of each behavioral test. Thus, each animal was separated from dam and siblings for about 4 min. Drugs were administered in such a way that the last open-field session was held during the dark phase of the cycle. The open field and the colony room were illuminated by a white lamp located in an adjacent room in order to minimize the effect of light on the cyrcadian rhythm of the rats.

Measurement of Catalepsy. Catalepsy was measured using a wire grid (20 x 25 cm) inclined 45 degrees relative to the bench top. Each rat was placed with its forepaws near the edge of the grid and the amount of time spent in this atypical position was recorded 3 times for each interval after drug injection (3, 6 or 9 h) using a stopwatch. Each rat was placed on the inclined grid and observed for a maximum of 60 s. If the animal did not move within 60 s it was removed and returned again to the grid. At the end of the three replications, the mean time spent by the rat without moving was calculated for each test. This technique was selected over the other alternative methods due to the fact that previous studies demonstrated its high sensitivity to neuroleptics (Campbell et al. 1984). The catalepsy scores were obtained each day at 3 h, 6h and 12 h after drug administration. Animals were treated as described in

the Open field behavior section and the last catalepsy test was carried out during the dark phase of the cycle.

Statistical Analysis. The data were first anallyzed by a 4-way ANOVA with the session (the open field test sessions lasted 3 min while catalepsy test session time varied depending on rat behavior) and day factors treated as within subject factor. A significant diet x dose x session x day, diet x dose x day, diet x day or dose x day interaction was observed for locomotion and catalepsy indicating that the behavioral response to drugs (chlorpromazine and haloperidol) was distinct on each day depending on the nutritional treatment and/or dose administered to young rats. Thus, in order to simplify data interpretation, a 3-way ANOVA was carried out on each day: 2 diets x 5 doses of chlorpromazine (or 6 doses of haloperidol) x 4 (sessions - for open-field and rearing data) or 3 (sessions - for catalepsy data) with the sessions factor treated as within subject factor. Data were analyzed using the SSPS/PC program and significant effects demonstrated by ANOVA were accepted only when the results of MANOVA were also significant (O'Brien and Kaiser 1985).

For the case of locomotor activity, additional statistical analysis was carried out in order to highlight whether the differences in response to the drugs were not related to differences in the baseline activity of normal and undernourished saline-treated groups. At each time point, the mean score of saline-treated rats in each nutritional group was used as a 100% and the values of the groups were calculated as the ratio between individual scores/mean score of respective saline group. Thus, in each nutritional group, locomotor activity was analysed as a percent of the respective saline-treated group.

Significant main effects or low level interactions were discussed only when higher order interactions were not statistically significant.

Results

Body Weight of Weaning rats

Undernourished animals (19.4 ± 1.2, mean ± SD for 35 litters used in the two experiments) present lower body weight than normal rats (43.8 ± 1.9 for 30 litters).

The effects of undernutrition and drugs on rearing response were essentially similar to those observed in crossing responses. Thus, to avoid redundant data presentation only crossing data will be described.

Chlorpromazine - Crossing - day 1 .

Analysis of between-subject effects for crossing on day 1 revealed a significant diet x dose interaction ($F(4,121)=4.09$, $p<0.01$) as a result of the higher total activity scores (sum of all sessions) of undernourished rats treated with saline (0 mg/kg), 2.5 and 5 mg/kg chlorpromazine, when compared to normal rats (data not shown).

Analysis of within-subject effects yielded a significant diet x dose x session interaction ($F(12,363)=2.34$, $p<0.01$), which was mainly due to the reduced response of undernourished animals to 2.5 mg/kg chlorpromazine at 4 h 30 min and to 2.5 and 5 mg/kg chlorpromazine at 7 h 30 min and 10 h 30 min after drug administration. The higher activity of saline-injected undernourished animals when compared to normal rats also contributed to this interaction (Figure 1A).

When data were analysed as a percent of saline-treated groups, ANOVA revealed similar between-subject and within-subject effects to those observed in the analysis using absolute scores (diet x dose interaction, $F(4,121)=2.57$, $p<0.05$; and a significant diet x dose x session interaction, $F(12,363)=2.03$, $p<0.05$). These results corroborate that undernourished rats responded less to 2.5mg/kg CPZ during session carried out at 4h 30min and to 2.5 and 5 mg/kg CPZ during sessions carried out at 7h 30min and 10h 30min after drug administration (Table 1) in spite to the increase in baseline activity of saline-treated undernourished rats.

Chlorpromazine- Crossing day 2 .

Analysis of between-subject effects of crossing responses on day 2 revealed a significant diet x dose interaction ($F(4,121)=3.59$, $p<0.05$) due to the higher activity of undernourished rats treated with 5 mg/kg chlorpromazine when compared with normal rats (data not shown).

Analysis of within-subject effects yielded a significant diet x session interaction ($F(3,363)=3.11$, $p<0.05$) as a consequence of the higher activity of undernourished rats injected with 5 mg/kg chlorpromazine when compared with normal rats at 4 h 30 min, 7 h 30 min and 10 h 30 min (Figure 1B).

When data were analysed as a percent of saline-treated groups, ANOVA revealed a significant diet x dose interaction ($F(4,121)=3.08$, $p<0.05$) and a significant diet x session interaction ($F(3,363)=4.43$, $p<0.01$). These interactions were significant mainly due to the fact that 5 mg/kg CPZ-treated rats reponded less to CPZ at 4h 30min to 10h 30min than did normal animals (Table 1)

These results showed that undernourished rats tend to respond less to CPZ on day 1 and 2, but this was more evident on day 1.

Chlorpromazine- Catalepsy day 1.

Analysis of between-subject effects revealed a main effect of dose ($F(4,121)=201.7$, $p<0.01$) as a result of the dose dependent increase in catalepsy scores (sum of the scores obtained during all sessions, data not shown).

nalysis of within-subject effects revealed a significant diet x dose x session interaction ($F(8,242)=2.90$, $p<0.05$). The third order interaction was significant because 2.5 mg/kg, 5 mg/kg and 10 mg/kg chlorpromazine-injected undernourished rats present lower catalepsy scores than normal rats only at 6 h after drug administration (Figure 2A).

Chlorpromazine - Catalepsy day 2.

Analysis of catalepsy scores on day 2 revealed a main effect of dose ($F(4,121)=95.13$, $p<0.01$) due to the increase in catalepsy scores as a function of dose (data not shown).

Analysis of within-subject effects revealed a significant diet x session ($F(2,242)=3.28$, $p<0.05$) and dose x session interaction ($F(8,242)=9.91$, $p<0.01$). Dose x session interaction was significant because the catalepsy scores of rats injected with 2.5, 5, 10 and 20 mg/kg chlorpromazine decreased as a function of sessions (Figure 2B). Diet x session interaction was significant mainly due to the lower catalepsy scores of 2.5 mg/kg chlorpromazine-injected undernourished animals at 6 hours after drug administration when compared to normal rats (Figure 2B). Thus, as observerd for locomotor activity, undernourished animals tended to respond less to CPZ on day 1 and 2, although this was more evident on day 1.

Haloperidol - Crossing day 1 .

nalysis of between subject effects yielded a significant main effect of diet ($F(1,102)=65.5$, $p<0.01$) and of dose ($F(5,102)=30.2$, $p<0.01$) due to the greater overall activity of undernourished animals when compared to normal rats and to the dose-dependent reduction in locomotor activity in both dietary groups, respectively (data not shown).

Analysis of within subject effects revealed a significant diet x dose x session interaction ($F(15,306)=2.80$, $p<0.05$). This effect was mainly due to the fact that haloperidol-treated undernourished rats crossed more squares than did normal rats at 4h 30 min, 7h 30 min and 10h 30 min after administration (Fig 3A). The higher activity of saline-injected undernourished rats also contributed to this interaction.

When data was analysed as a percent of saline-treated groups, the statistical analysis revealed results similar to those obtained using the absolute scores. ANOVA reaveled a significant main effect of diet

($F(1,102)=4.21$, $p<0.05$) due to the greater overall activity of undernourished rats when compared with normal rats. ANOVA also revealed a significant main effect of dose ($F(5, 102)=4.47$, $p<0.01$) as a consequence of the dose-dependent reduction in locomotor activity of rats of both dietary groups. Analysis of within-subject effects revealed a significant diet x session ($F(3, 306)=3.34$, $p<0.05$) and a significant dose x session ($F(15, 306)=2.11$, $p<0.05$) as a consequence of reduced responsiveness of undernourished rats to haloperidol at 4h 30min to 10h 30min after drug administration when compared to normal animals (Table 2).

Haloperidol - Crossing day 2 .

Analysis revealed a significant main effect of diet ($F(1,102)=14.5$, $p<0.01$) due to the greater activity of undernourished animals when compared to normal rats and a significant main effect of dose ($F(4,102)=6.4$, $p<0.01$) due to the dose-dependent decrease in locomotion as a function of haloperidol dose (data not shown).

Analysis of within subject effects yielded a significant diet x session interaction ($F(3,306)=2.04$, $p<0.05$) due to the greater activity of 0.25-, 0.5-, 1- and 2 mg/kg haloperidol-injected undernourished rats when compared to normal rats during the sessions carried out at 7 h 30 min and 10 h 30 min (Figure 3B).

When the data were analysed as a percent of saline-treated groups, ANOVA yielded a significant main effect of diet ($F(1,102)=6.98$, $p<0.01$) and of dose ($F(5,102)=5.85$, $p<0.01$) as was observed when the data were analysed using the absolute values of locomotor activity. Analysis of within-subject effects yielded a significant diet x dose x session interaction ($F(15,306)=2.89$, $p<0.01$). This was due to the greater activity of 1- and 2 mg/kg haloperidol-treated undernourished rats when compared to normal rats during the session carried out from 4h 30min to 10h 30min. The greater activity of 0.25- and 0.5 mg/kg haloperidol-treated rats during the session carried out 7h 30min. Thus,

undernourished rats tend to respond less to haloperidol than nomal rats on days 1 and 2.

Haloperidol - Catalepsy - day 1.

Analysis of catalepsy data revealed a significant diet x dose interaction ($F(5,102)=3.88$, $p<0.01$) due to the overall reduced responsiveness of undernourished rats to 0.25, 0.5, 1 and 2 mg/kg haloperidol (data not shown).

Analysis of within subject effects revealed a significant dose x session ($F(10,204)=9.0$, $p<0.01$) and diet x session interaction ($F(2,204)=11.6$, $p<0.01$). These interactions were significant because the effect of haloperidol in both dietary groups decreased as a function of time, but the decrease was faster in undernourished animals. This was evident 6 h after drug administration (Figure 4A).

Haloperidol - Catalepsy - day 2.

Analysis of between subject effects yielded a significant diet x dose interaction ($F(5,102)=5.57$, $p<0.01$) due to the overall reduced sensitivity of undernourished animals to 0.25, 0.5 and 2 mg/kg haloperidol (data not shown).

Analysis of within subject effects revealed only a significant dose x session interaction ($F(10,204)=7.21$, $p<0.01$) due to the decrease responsiveness to haloperidol as a function of time in both dietary groups (Fig 4B). Thus as observed for locomotor activity, undernourished rats tended to respond less to haloperidol, but this was more evident on day 1.

Discussion

Undernutrition affected the motor activity of young rats on day 1 of testing as evidenced by the fact that saline-treated undernourished rats ambulated more than normal rats in sessions carried out at 4 h 30 min, 7 h 30 min and 10 h 30 min after saline administration. On day 1, undernourished animals tended to respond less to chlorpromazine and haloperidol (after 4 h 30 min) and this reduced sensitivity last for the rest of the observations that day. On day 2, the reduced sensitivity to chlorpromazine was evident only for animals injected with 5 mg/kg of the drug. For haloperidol, this was apparent for rats treated with 0.25, 0.5, 1 and 2 mg/kg. Similarly to the locomotor activity, catalepsy data indicated that early undernourished rats tended to show an overall hyposensitivity to chlorpromazine and haloperidol at 6 hours after drug administration on day 1. Since at an early time (3 hours), undernourished rats responded to chlorpromazine and haloperidol like normal rats it is reasonable to suppose that the drugs were eliminated more rapidly in undernourished animals. On day 2, the influence of undernutrition was not so clear. Several factors may contribute to the reduced response of undernourished animals to neuroleptics including alteration in distribution, uptake and elimination of the drugs. Changes in pharmacokinetic parameters seem plausible, since chlorpromazine and haloperidol are lipophilic drugs and early malnourished animals present less fat in their bodies (Smart et al. 1989). However, there are a considerable body of data showing that adipose tissue storage is not simply a matter of lipid solubility and partition. Functional groups of the drug molecules play a decisive role and basic lipophilic drugs such as haloperidol and chlorpromazine are not redistributed into adipose tissue (Betschart et al. 1988; Bickel, 1984; Moor et al. 1992). Therefore, adipose tissue may play a role only if these drugs were stored in the adipose tissue at the site of injection as observed by Betschart et al (1988) when they administered basic lipophilic drugs by the intraperitonial route. In this case, when the drugs are given via the intraperitonial route, the storage appears to

result from non-systemic, diffusional uptake from the peritoneal cavity (Betschart et al. 1988). Another factor that may have contributed to differences in the sensitivity to haloperidol and CPZ in undernourished rats is the fact that lipophilic drugs are transported bound to albumin in plasma (Goodman & Gilman's, 1996; Sato & Koshiro, 1995) and competition between antipsychotic drugs and other hydrophobic molecules may occur (Sato & Koshiro, 1995). Young undernourished animals presents higher levels of free fatty acids in plasma than does normal rats (Morgane et al. 1978). Thus, it is plausible that free fatty acids compete with CPZ and haloperidol for binding to albumin which may alter the distribution and elimination of these drugs in undernourished animals.

Acute administration of neuroleptics such as chlorpromazine and haloperidol causes an increase in dopamine turnover, as measured by increased tyrosine hydroxylase activity and dihydroxy-phenylacetic acid and homovanilic acid concentrations (Bannon et al. 1983; Carlsson and Lindqvist 1963; Coyle et al. 1985; Ericson et al.. 1996; Fekete and Borsy 1971; Kolenik et al. 1989; Nyback and Sedvall 1969). Thus, it is possible that some of the doses of chlorpromazine and haloperidol used in the present investigation changed the metabolism of dopamine in undernourished animals in a such way that the reduced sensitivity observed on day 1 decreased on day 2. Alternatively, there is also evidence suggesting that the behavioral and biochemical effects of dopaminergic agents are influenced by novelty (Bardo et al. 1990). In the present report, young undernourished rats showed hyperactivity in the open-field on the first day of exposure, which may indicate that they interact with novelty in a different way than do normal rats. Previous published data demonstrated that early undernourished adult rats react behaviorally and neurochemically to novel situations in a different way than do normal rats (Mello et al. 1989; Perry et al. 1990; Rocha & Mello 1994; Vendite et al. 1988; Vendite et al. 1990). Thus, it is possible that the hyperactivity observed in undernourished rats contributed to the behavioral hyposensitivity

to chlorpromazine and haloperidol. However, the difference between saline-treated rats in the two groups at all but the first time point (1h 30 min) on the first day could also be explained by a faster habituation of normal rats to the apparatus.

In summary, the results of the present study demonstrated that young undernourished rats are hyposensitive to various doses of chlorpromazine and haloperidol in two classes of behavior. The fact that the effect of neuroleptics (mainly on day 1) on locomotion and catalepsy were similar for the two groups at the first time point and then became less pronounced in undernourished rats as a function of time point to changes in the pharmacokinetics of chlorpromazine and haloperidol in undernourished animals. However, additional investigations are necessary to clarify whether changes in brain sites are also involved in the observed hyposensitivity, since previous studies have demonstrated that weaning undernourished rats showed low density of D2 sites in the striatum.

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LEGEND TO FIGURES

FIGURE 1 - Effect of chlorpromazine on locomotor activity of young rats on day 1 (panel A) and 2 (panel B). Blank squares (normal rats); filled squares (undernourished rats). N=12 to 14 rats in each group. Data are expressed as mean±S.E.M.

FIGURE 2 - Effect of chlorpromazine on catalepsy scores of young rats on day 1 (panel A) and 2 (panel B). Blank squares (normal rats); filled squares (undernourished rats). N=12 t o 14 rats in each group. Data are expressed as mean±S.E.M.

FIGURE 3 - Effect of haloperidol on locomotor activity of young rats on day 1 (panel A) and 2 (panel B). Blank squares (normal rats); filled squares (undernourished rats). N= 9 to 11 rats in each experimental group . Data are expressed as mean±S.E.M

FIGURE 4 - Effect of haloperidol on catalepsy scores of young rats on day 1 (panel A) and 2 (panel B). Blank squares (normal rats); filled squares (undernourished rats). N= 9 to 11 rats in each experimental group. Data are expressed as mean±S.E.M.

Table 1 - Effects of undernutrition during suckling on the behavioral response to chlorpromazine of weaning rats (% locomotor activity of saline-treated group).

Time after injection				
1h 30 min	DAY 1		DAY 2	
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±8.0	100.0±6.6	100.0±7.8	100.0±8.2
2.5	57.7±4.6	37.4±3.0*	72.0±6.7	52.6±7.2
5	14.3±1.8	9..4±2.2	26.8±2.3	38.9±4.4
10	3.2±0.9	1.5±0.4	18.0±4.2	3.9±1.2
20	0.7±0.2	1.3±0.6	10.4±3.1	2.0±1.2
4 h 30 min				
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±7.8	100.0±7.3	100.0±11.8	100.0±12.8
2.5	58.9±4.1	78.9±5.2*	78.3±10.9	56.6±7.2
5	24.9±3.6	27.9±3.1	7.8±1.6	68.6±10.4*
10	4.3±1.9	1.7±0.8	17.2±5.5	9.1±4.1
20	1.0±0.6	0.0±0.0	6.4±1.7	4.7±1.0
7 h 30 min				
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±11.3	100.0±8.7	100.0±13.9	100.0±13.7
2.5	44.5±7.9	133.4±11.8*	84.4±8.8	94.7±10.8
5	25.6±5.7	58.9±5.0*	10.5±2.3	121.6±14.4*
10	18.1±2.8	13.4±1.9	20.0±4.8	43.5±6.3*
20	0.7±0.3	0..1±0.3	0.0±0.0	7.4±2.4
10 h 30 min				
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±9.6	100.0±8.8	100.0±8.2	100.0±10.8
2.5	40.1±4.7	107.4±9.1*	100.3±12.9	110.6±11.1
5	41.7±5.8	90.9±7.8*	18.5±3.7	97.2±10.7*
10	25.6±3.1	26.7±3.3	34.5±4.9	32.9±5.8
20	14.9±2.8	8.5±2.5	16.2±3.5	35.6±6.7

*Indicates a significant difference from normal group within the same day ($p<0.05$; comparisons by Duncan's Mutiple Range test). Data are expressed as mean±S.E.M.

Table 2 - Effects of undernutrition during suckling on the behavioral response to haloperidol of weaning rats (% locomotor activity of saline-treated group).

Time after injection
1h 30 min

	DAY 1		DAY 2	
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±6.0	100.0±5.9	100.0±8.9	100.0±10.5
0.125	38.5±2.8	44.1±5.0	58.7±5.3	75.5±7.7
0.25	1.8±0.3	4.0±0.7	30.3±4.0	28.5±3.9
0.5	0.6±0.2	3.6±1.0	23.0±1.5	33.6±3.5
1	0.0±0.0	1.5±0.4	25.3±5.9	14.5±2.8
2	2.1±0.7	0.3±0.3	2.9±0.4	6.1±1.8

4 h 30 min

	DAY 1		DAY 2	
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±5.7	100.0±5.9	100.0±10.3	100.0±11.7
0.125	57.0±7.5	63.3±4.8	58.3±6.3	79.8±6.8
0.25	2.0±0.6	40.8±3.2*	34.5±5.6	39.2±5.0
0.5	0.0±0.0	26.2±3.4*	20.3±3.9	33.5±2.8
1	0.8±0.2	12.3±1.7*	2.5±0.3	63.7±8.2*
2	0.0±0.0	0.6±0.4	12.0±1.4	66.4±9.1*

7 h 30 min

	DAY 1		DAY 2	
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±10.4	100.0±6.2	100.0±6.8	100.0±8.9
0.125	105.0±9.1	113.4±6.8	55.1±4.4	89.4±9.3
0.25	61.6±6.5	116.4±9.4*	43.6±4.2	98.8±10.6*
0.5	66.7±7.7	54.8±3.9	27.6±3.6	75.0±8.4*
1	12.8±1.7	50.0±3.9*	15.4±1.9	71.0±9.1*
2	0.0±0.0	17.3±1.1*	16.3±2.1	76.8±5.6*

10 h 30 min

	DAY 1		DAY 2	
DOSE (mg/kg)	normal	undernourished	normal	undernourished
0	100.0±8.2	100.0±7.9	100.0±9.8	100.0±6.7
0.125	111.6±6.8	116.8±4.0	144.4±10.9	57.2±5.1*
0.25	71.9±6.7	74.3±5.3	53.9±4.8	58.6±6.3
0.5	74.8±7.4	83.7±7.2	73.1±5.7	69.4±6.9
1	28.4±3.9	85.6±4.8*	43.8±4.5	80.7±6.8*
2	17.2±1.9	52.0±3.9*	23.8±2.8	70.1±4.2*

*Indicates a significant difference from normal group within the same day ($p<0.05$; comparisons by Duncan's Mutiple Range test).

Day 1 - Crossing

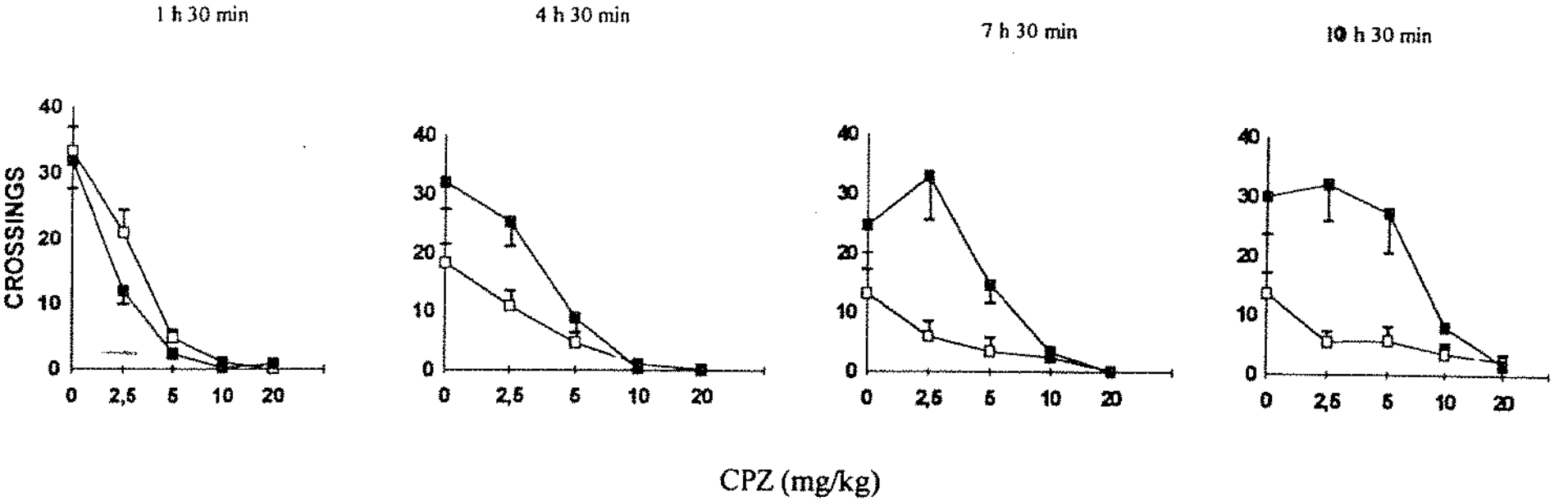

Figure 1.A

Day 2 - Crossing

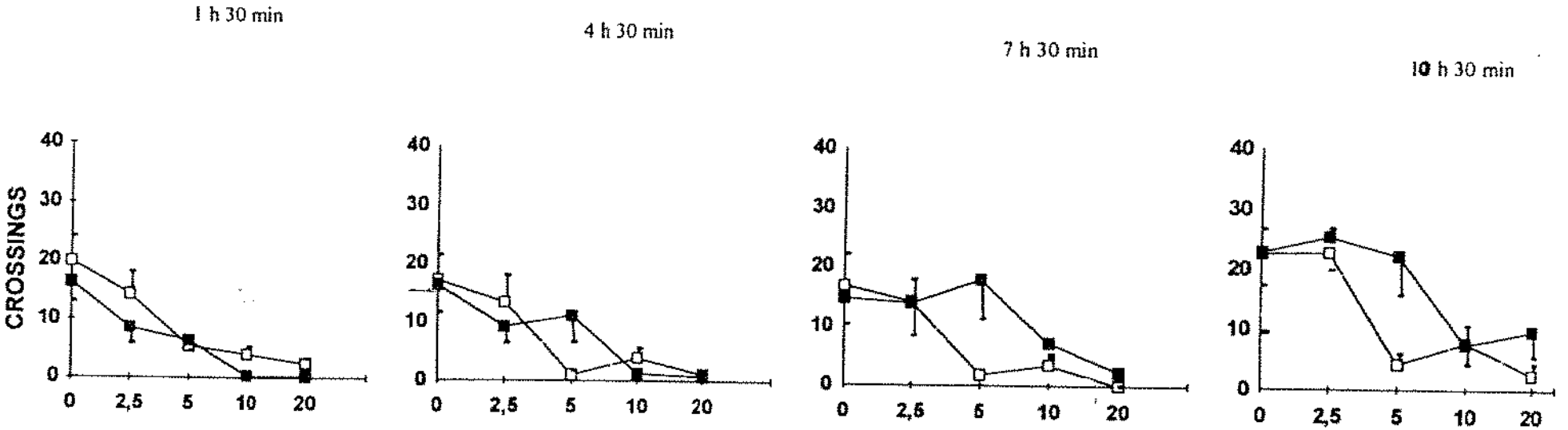

Figure 1.B

Day 1 - Catalepsy

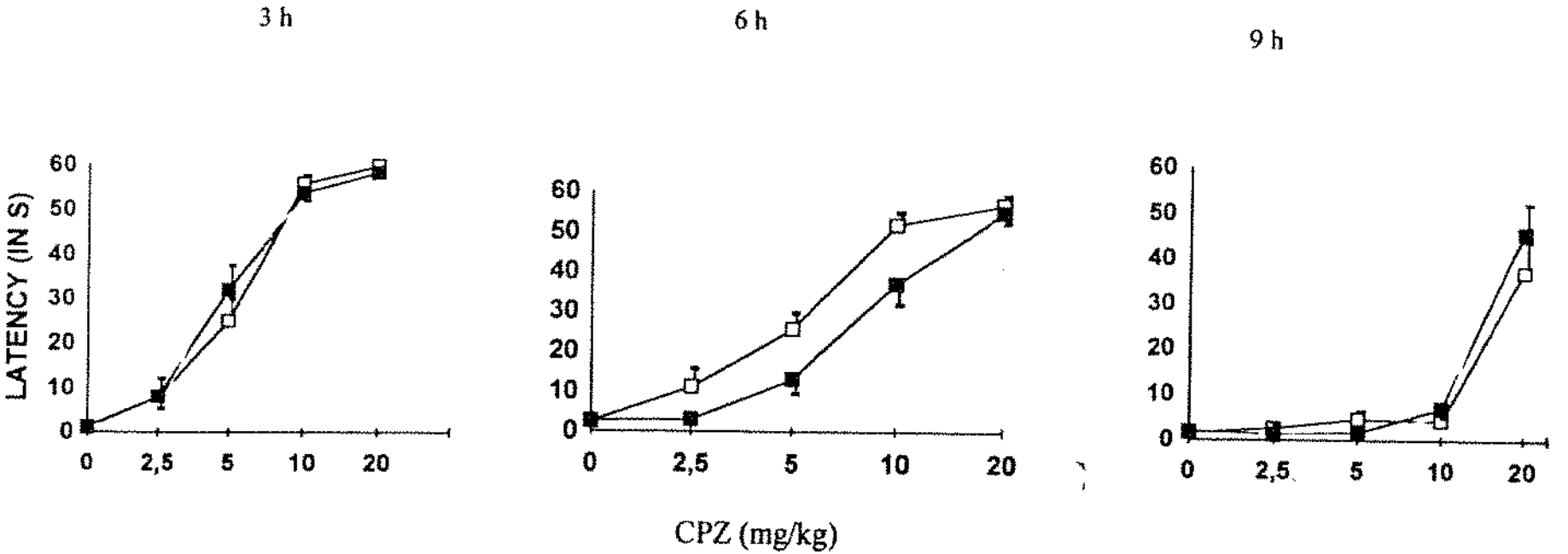

Figure 2.A

Day 2 - Catalepsy

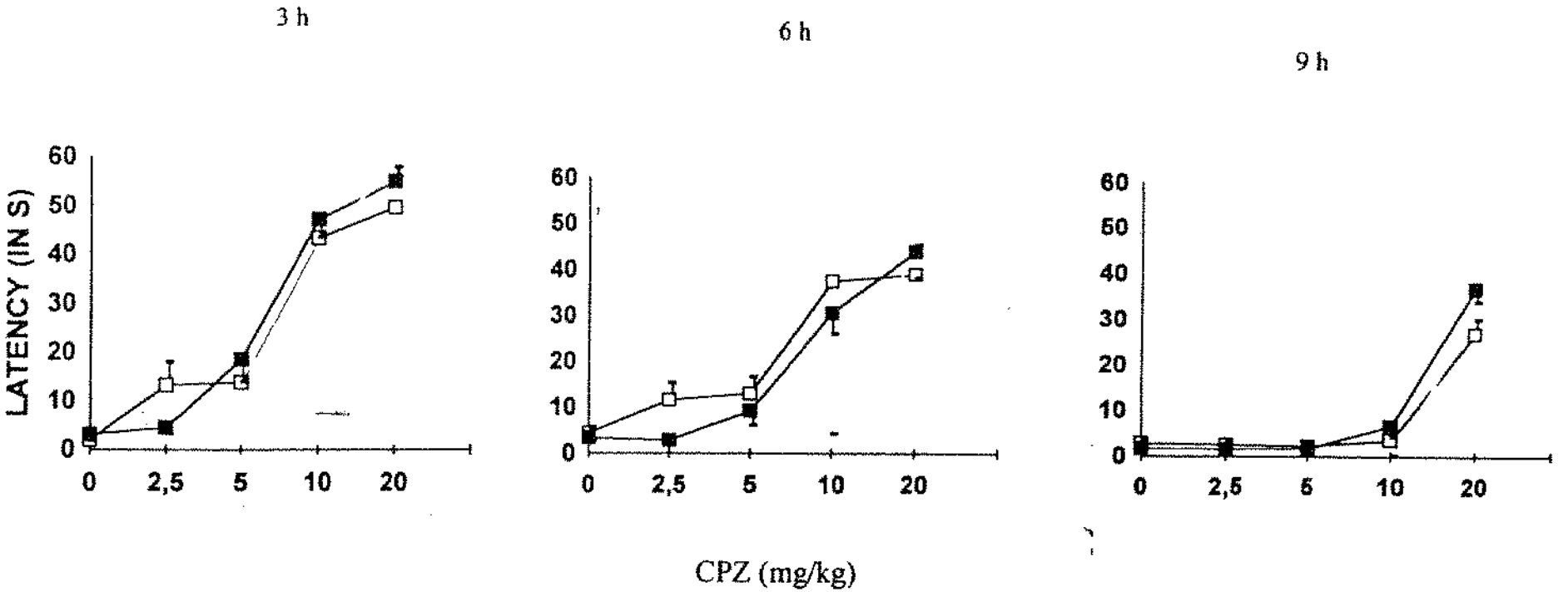

Figure 2.B

Day 1 - Crossing

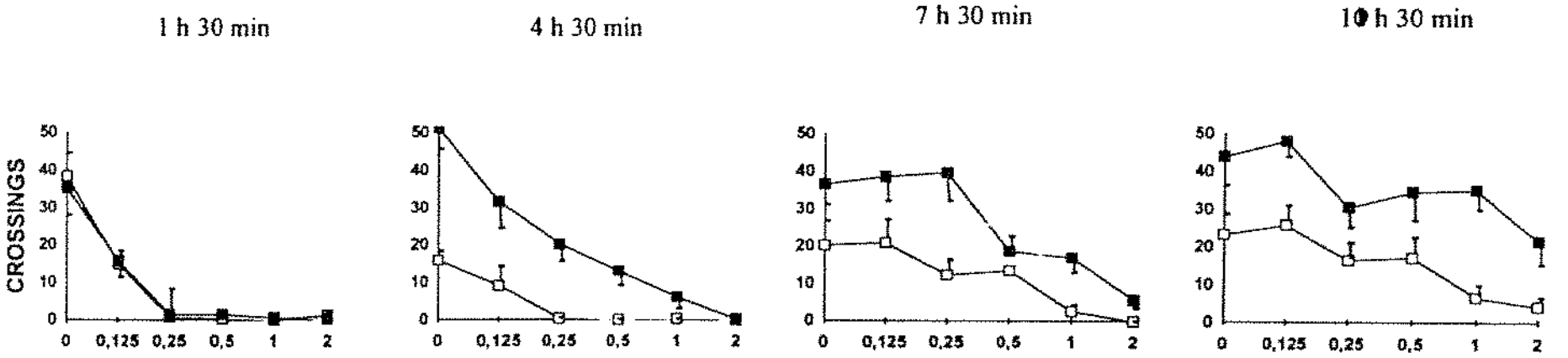

Figure 3.A

Day 2 - Crossing

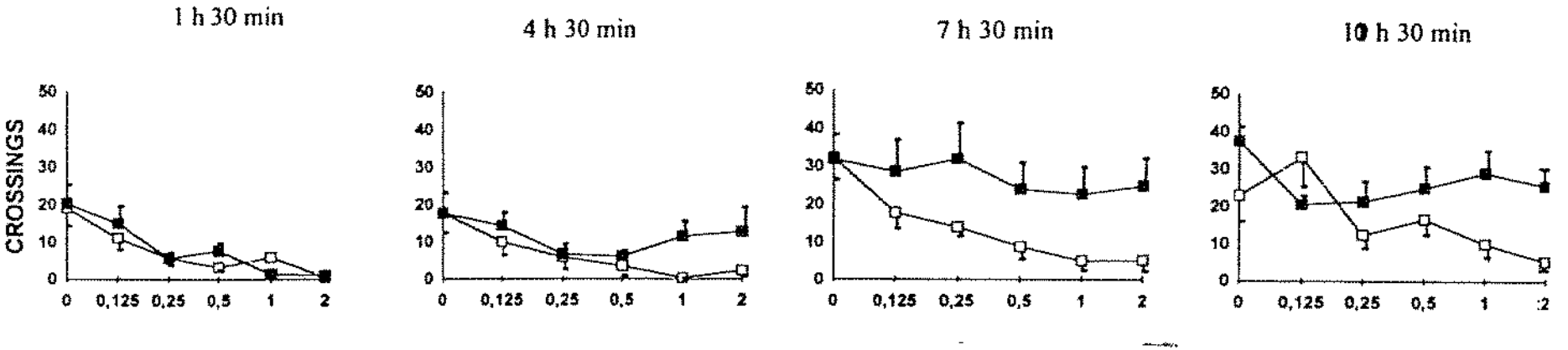

Figure 3.B

Day 1 - Catalepsy

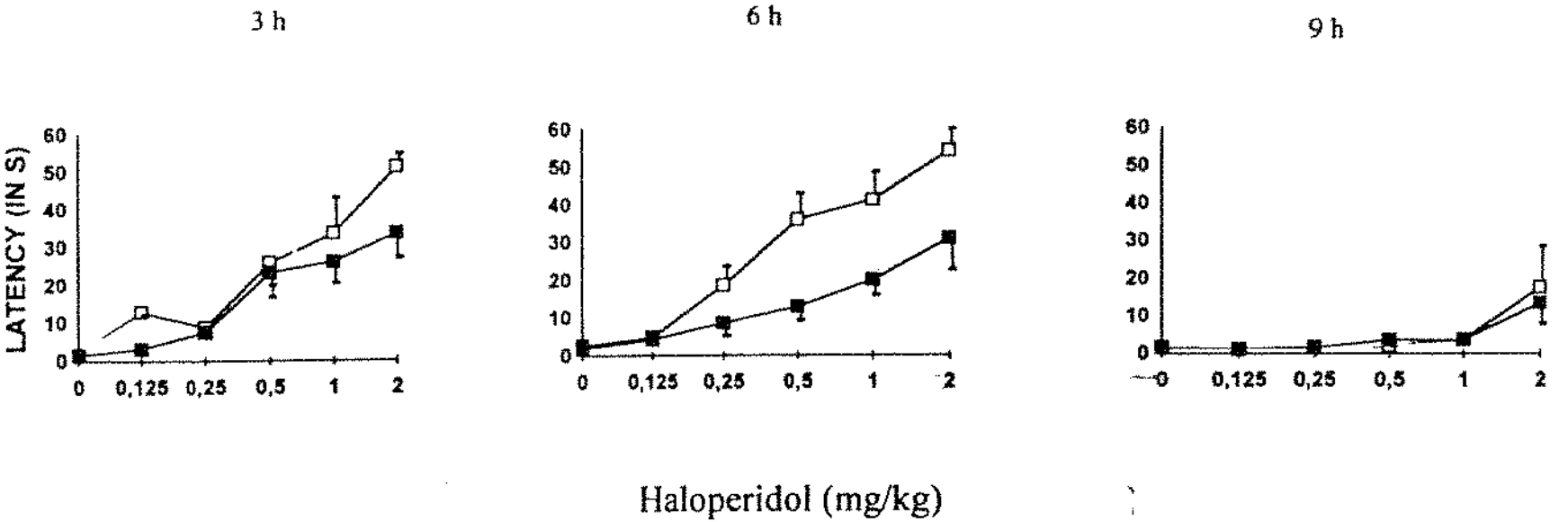

Figure 4.A

Day 2 - Catalepsy

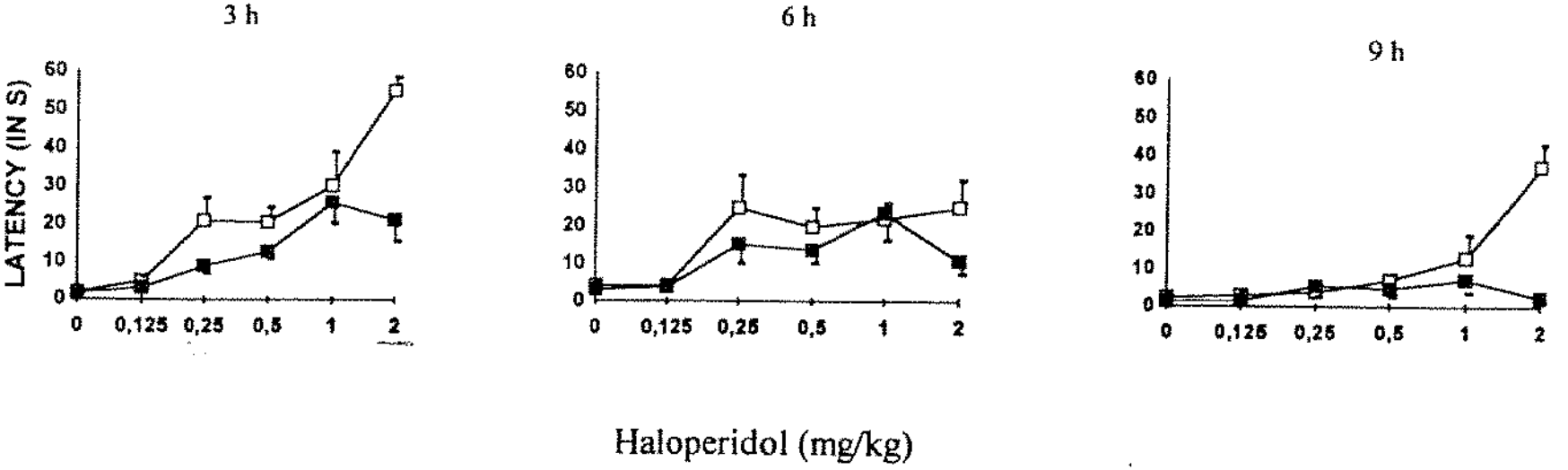

Figure 4.B

EFFECTS OF UNDERNUTRITION DURING SUCKLING ON THE BEHAVIORAL RESPONSES OF WEANLING RATS TO SCH23390

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Keywords: Undernutrition, catalepsy, locomotor activity, SCH23390.

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Summary:

Undernutrition during critical periods of development may change the behavioral responses of rats to centrally acting drugs. In the present study, the effects of undernutrition during suckling on the behavioral responses of 21-days-old rats to SCH23390 (0, 0.3, 0.6 and 1.2mg/kg) were examined. Locomotion and catalepsy were assessed at 25 min, 1 h 10 min, 1 h 55 min and 2 h 40 min after drug administration on two consecutive days. SCH23390 induced a similar inhibition of locomotor activity in both normal and undernourished animals. On the other hand, SCH23390-induced catalepsy was reversed differently in undernourished animals compared to normal animals with resulting lower catalepsy scores at the 1 h 55 min and 2 h 40 min assessments for the undernourished animals on day 1, but not on day 2. It is suggested that the behavioral effects of SCH 23390 are less persistent in undernourished rats, possibly due to a change in drug pharmacokinetics, when compared to well-nourished rats.

Introduction: Undernutrition during early periods of development may cause permanent alterations in the neurochemistry and behavior of experimental animals (1,2). Various studies have demonstrated that perinatal undernutrition causes transitory or permanent changes in enzyme activities related to various neurotransmitter systems as well as changes in the levels of various classes of neurotransmitters (2,3).

Several other studies have demonstrated that animals undernourished during pregnancy and/or suckling present alterations in response to drugs acting on the central nervous system during adulthood (4). In contrast, only a limited number of studies have investigated the effect of drugs in young rats (5). Investigation of drugs that exert their effects centrally in developing rats is of considerable interest, since undernourished young rats may present changes in the levels of nor-epinephrine, serotonin and dopamine. (2).

In a previous study, we have shown that undernourished weanling rats were hyposensitive to chlorpromazine and haloperidol as evaluated by catalepsy and spontaneous locomotor activity(5). These two classes of behavior are presumably controlled by different pathways of the dopaminergic system (6-9). A change in drug pharmacokinetics associated with undernutrition was suggested as a possible explanation for the differences observed(5).

Since undernourished rats showed similarly attenuated responses to chlorpromazine (a D1 and D2 antagonist) and haloperidol (a D2 antagonist) when compared to normal rats (5), we decided to investigate whether SCH23390, a D1 antagonist with cataleptogenic effects analogous to those of other neuroleptics but with a much shorter duration of action (10), would lead to comparable results.

Materials and Methods

Undernutrition. Wistar rats from our own breeding stock were used. Rats were maintained on a 12 h light/12 h dark cycle (lights on at 7 a.m.). On the day of birth (day 0), litters were adjusted to 9 pups and half the dams were assigned at random to one of the nutritional groups. Normal dams had free access to a commercial diet,

while undernourished dams were on a restricted food regimen. On day 0, food was removed and thereafter the following food scheme was applied to the undernourished dams: from day 1 to 7 they received approximately 12 g of food/day, from day 8 to 14 approximately 17 g, and from day 15 to 22 approximately 25 g/day. Water was available at all times to rats assigned to both nutritional treatments.

SCH23390 treatment: On day 20, offspring of both dietary groups were weighed and randomly assigned to one of the following doses of SCH233390: 0 (saline treated group), 0.3, 0.6, and 1.2 mg/kg. On day 21, rats were weighed and injected subcutaneously with SCH23390 (10 ml/kg body weight). Twenty four hours after the first drug administration (day 22), rats were weighed and injected with the same dose as used on day 1. Rats within a litter were assigned at random to a given dose but in such a way that no more than one rat from a single litter was assigned to the same dose. Rats were returned to their home cage immediately after each injection.

Open field behavior and Measurement of Catalepsy: Locomotion was assessed by the number of squares crossed with the four paws in an open-field arena measuring 56 x 42 x 40 cm (high) whose floor was divided into 12 squares. Each open-field session lasted 2 min and was carried out 25 min, 1 hr 10 min, 1 hr 55 min, and 2 hr 40 min after each daily injection. Catalepsy was measured immediately after each open-field test using a wire grid (20 x 25 cm) inclined 45 degrees relative to the bench top. Each rat was placed with its forepaws near the edge of the grid and the amount of time spent in this atypical position was recorded 3 times. Each rat was placed on the inclined grid and observed for a maximum of 60 s. If the animal did not move, it was removed from the grid and then returned to it. If it did not move within 60 s, it was again removed and returned to the grid. At the end of the three replications, the mean time spent by the rat without moving was calculated for each test.

Statistical Analysis: In order to simplify data interpretation, a 3-way ANOVA was carried out on each day: 2 diets x 4 doses of SCH23390 x 4 (sessions). Significant

main effects or low level interactions were discussed only when higher order interactions were not statistically significant.

Results and Discussion: Body Weight of Weaning rats. Undernourished animals (21.4 ± 1.8, mean ± SD for 22 litters) present a lower body weight than normal rats (41.2 ± 2.7 for 17 litters).

On day 1, SCH23390 administration promoted a significant decrease in locomotor activity ($p<0.001$); however, no significant effect of dietary treatment was observed. The inhibitory effect of SCH23390 on locomotor activity decreased as a function of time, as demonstrated by the significant dose x time interaction ($p<0.001$), regardless of the nutritional group (Figure 1a). The locomotor activity of saline-treated animals decreased as a function of sessions in both dietary groups. On day 2, the behavioral effects of SCH23390 were essentially similar to those observed on day 1 (Figure 1b).

Catalepsy results demonstrated that SCH23390 produced a dose-dependent increase in catalepsy scores on the two days of testing for both normal and undernourished animals ($p<0.001$). The cataleptogenic effect of SCH23390 decreased with time, but on day 1 the catalepsy scores were reversed faster in the undernourished group than in normal rats, as demonstrated by a significant diet x session ($p<0.005$) and a dose x session interaction ($p<0.001$).

The present results show that undernutrition during suckling did not affect the SCH23390-induced reduction in spontaneous locomotor activity of weanling rats; however, the cataleptogenic effect of the drug was less persistent in undernourished animals than in normal rats after the first injection of the compound. This effect of SCH23390 on catalepsy was somewhat similar to those previously observed for chlorpromazine and haloperidol (5), two classical neuroleptic agents. The fact that SCH23390 initially (25 to 70 min after injection) produced similar catalepsy scores for both nutritional groups and lower scores for undernourished animals during the third and fourth sessions may indicate that SCH23390 is eliminated more rapidly in undernourished than in normal animals. The absence of such an effect on locomotor

activity may be related to the fact that catalepsy and locomotor activity are regulated by different dopaminergic pathways (11). Catalepsy seems to be related to extrapyramidal effects of neuroleptics (7) and locomotion seems to be associated with pathways located in other brain regions such as the mesocorticolimbic dopamine system (8,9).

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LEGEND TO FIGURES

FIGURE 1 - Effect of SCH23390 on locomotor activity of young rats on day 1 (panel A) and 2 (panel B). Blank squares (normal rats); filled squares (undernourished rats). N=15 to 20 rats in each group. Data are expressed as mean±S.E.M.

FIGURE 2 - Effect of SCH23390 on catalepsy scores of young rats on day 1 (panel A) and 2 (panel B). Blank squares (normal rats); filled squares (undernourished rats). N=15 to 20 rats in each group. Data are expressed as mean±S.E.M.

Day 1 - Crossing

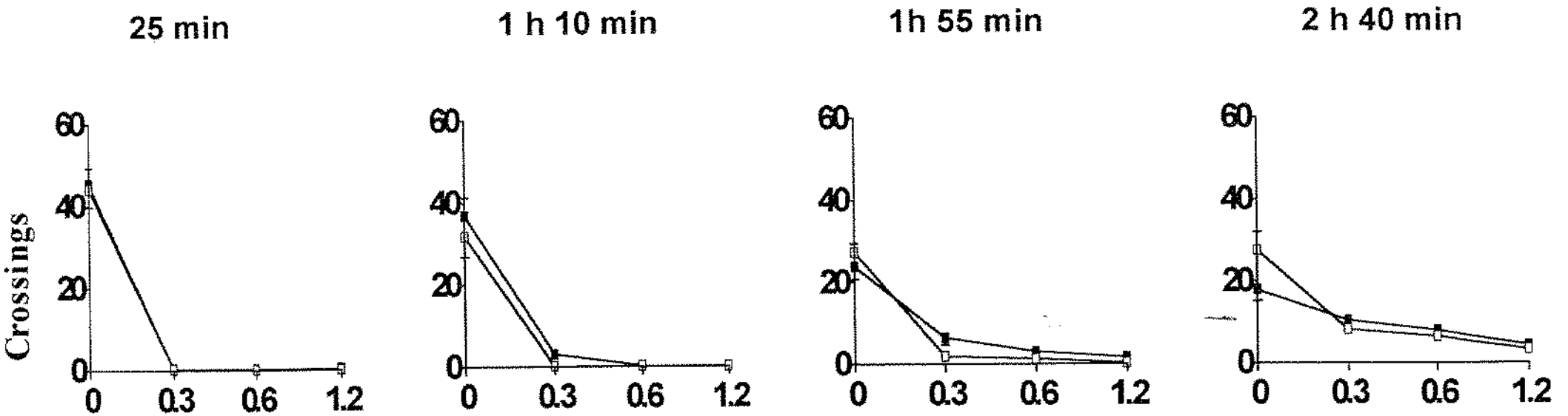


Figure 1.A

Day 2 - Crossing

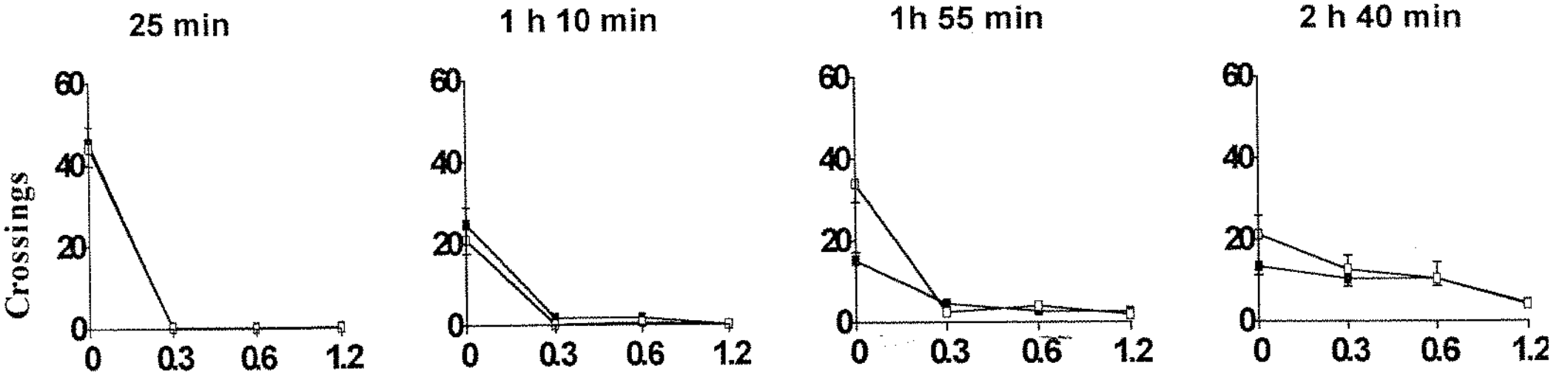


Figure 1.B

Day 1 - Catalepsy

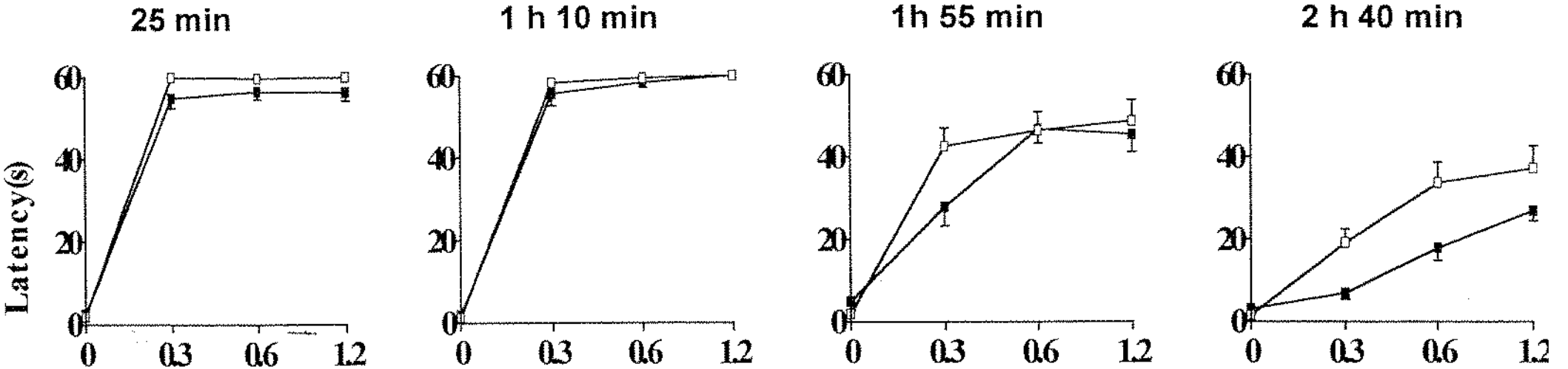
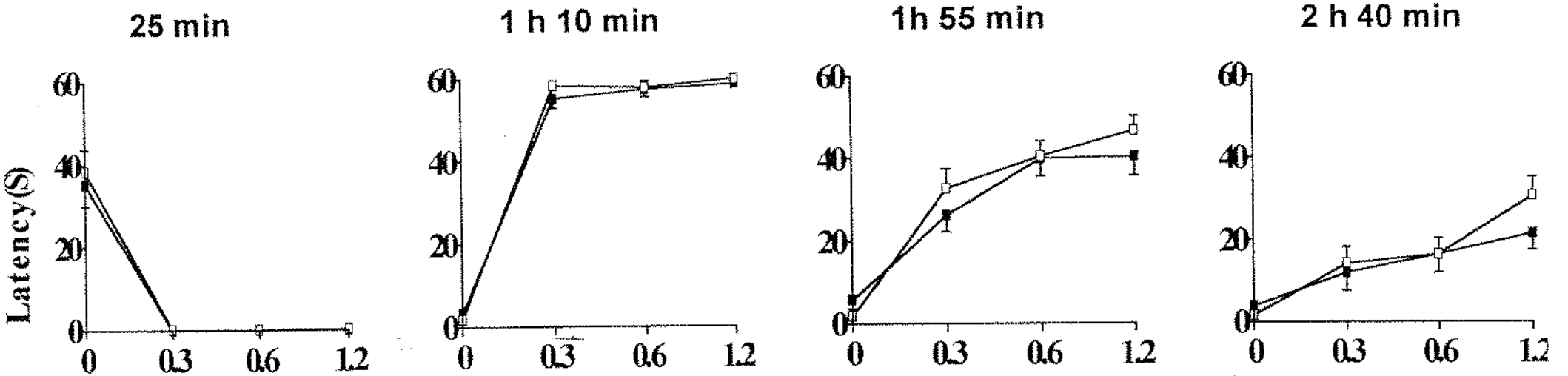


Figure 2.A

Day 2 - Catalepsy



SCH23390 (mg/kg)

Figure 2.B

DISCUSSÃO

A desnutrição afetou, isoladamente, a atividade locomotora dos ratos jovens no primeiro dia de teste. Isto ficou evidenciado pelo fato de os animais desnutridos tratados com salina terem apresentado atividade locomotora mais intensa do que os animais normais, notadamente a partir da segunda sessão de atividade locomotora. No primeiro dia de tratamento, os animais desnutridos mostraram respostas menos intensas à clorpromazina e ao haloperidol, a partir da segunda sessão de campo aberto. Esta diferença persistiu nas outras sessões do mesmo dia. No segundo dia de tratamento, os resultados mostraram diferenças menos evidentes entre os animais normais e desnutridos, porém houve uma tendência a respostas menos intensas à clorpromazina e ao haloperidol, para os ratos desnutridos. Já o SCH23390 não produziu efeitos diferentes nos animais normais, quando comparados com os desnutridos, no que se refere a atividade locomotora, em ambos os dias de tratamento.

Quanto à catalepsia induzida por estas drogas, observou-se que os ratos desnutridos apresentaram menor sensibilidade aos efeitos cataleptogênicos da clorpromazina, haloperidol e SCH23390, nitidamente no primeiro dia de tratamento. Porém a hipossensibilidade dos animais desnutridos ao haloperidol e clorpromazina não foi notada na primeira sessão de avaliação da catalapsia (3 h após administração do haloperidol ou clorpromazina), somente tendo ocorrido a partir da segunda sessão. Como os animais desnutridos sofreram efeitos de mesma intensidade que os observados nos animais normais, inicialmente, porém recuperaram-se destes efeitos mais rapidamente, parece sustentável supor que as drogas tenham sido eliminadas mais rapidamente nos animais desnutridos. O mesmo aconteceu ao testarmos o SCH23390, pois os animais desnutridos responderam de maneira idêntica aos animais normais, inicialmente, porém recuperaram-se mais rapidamente do que os animais normais, em relação aos efeitos cataleptogênicos do SCH23390. No segundo dia de tratamento, as diferenças entre os grupos foram menos evidentes.

Muitos fatores podem ter contribuído para a menor sensibilidade dos animais desnutridos aos efeitos destas três drogas, principalmente os fatores farmacocinéticos. A clorpromazina e o haloperidol são drogas muito lipossolúveis, portanto poderiam sofrer grande influência da menor quantidade de tecido adiposo presente nos animais desnutridos (Smart et al. 1989). Entretanto, alguns estudos demonstraram que o acúmulo de uma droga no tecido adiposo não é, simplemente, determinado pela sua lipossolubulidade e coeficiente de partição. Alguns grupos funcionais presentes na estrutura molecular da droga desempenham papéis decisivos neste sentido. Assim, drogas básicas, embora lipofílicas, tais como a clorpromazina e o haloperidol, não são prontamente distribuídas para o tecido adiposo (Betschart et al. 1988; Bickel, 1984; Moor et al, 1992).

Estudos da farmacocinética do haloperidol e da clorpromazina em humanos mostraram, para ambas as drogas, grandes volumes de distribuição, superando em muito o volume corporal, pois resultaram em cerca de 20 litros/kg de peso corporal (Goodman & Gilman's, 1996). Na verdade, estes grandes volumes de distribuição refletem a intensa ligação destas drogas a sítios fora do compartimento plasmático. Estudos de farmacocinética destas drogas em ratos são raros, porém um trabalho avaliou o padrão de distribuição tecidual do haloperidol, o qual foi utilizado em doses altas, por um período de até três semanas. Curiosamente, as maiores concentrações teciduais da droga foram encontradas nos pulmões; as menores, foram encontradas nos músculos e tecido adiposo (Moor et al. 1992). Este estudo mostra que, mesmo sob as condições mais favoráveis, o haloperidol não se acumula no tecido adiposo, apesar da sua grande lipossolubilidade.

Daniel et al.(1997) confirmaram estes achados estudando drogas psicotrópicas lipossolúveis. Observaram que estas drogas, por serem muito lipossolúveis, porém básicas, são acumuladas em compartimentos subcelulares, principalmente nos lisossomas. Por se tratarem de bases fracas, suas porções não-ionizadas cruzam membranas, dependendo do pH extracelular, terminando por acumularem-se no interior dos lisossomas, onde

se combinam com prótons, o que impede a sua difusão no sentido inverso. Estas drogas também se ligam, de forma não específica, a fosfolipídios de membrana. Ainda, as maiores concentrações destas drogas foram observadas nos pulmões, os quais contêm grandes quantidades de macrófagos alveolares (ricos em lisossomas) e surfactante pulmonar (uma mistura complexa de proteínas e fosfolipídios)(Daniel et al. 1997).

Por outro lado, o tecido adiposo poderia desempenhar um papel fundamental em casos particulares, tais como no estudo realizado por Betschart et al (1988), no qual drogas lipofílicas foram injetadas na cavidade peritoneal. Neste caso, o acúmulo das drogas no tecido adiposo parece ter resultado da simples difusão das mesmas, a partir da cavidade peritoneal, para a gordura extra-peritoneal, ou seja, de forma não-sistêmica (Betschart et al. 1988).

Outro fator que pode ter contribuído para a hipossensibilidade dos animais desnutridos às drogas testadas é o fato de as mesmas serem transportadas no plasma, intensamente ligadas à albumina (Goodman & Gilman's, 1996; Sato & Koshiro, 1995). Os animais desnutridos podem ter menores concentrações plasmáticas de albumina, além de apresentarem maiores níveis plasmáticos de ácidos graxos livres(Morgane et al. 1978), os quais competem com antipsicóticos e outras moléculas hidrofóbicas (Sato & Koshiro, 1995) pela albumina. Portanto, parece muito provável que um aumento dos ácidos graxos livres nos animais desnutridos possa ter resultado em maiores quantidades de droga livre e, conseqüentemente, maior velocidade de eliminação das mesmas.

O tratamento agudo com neurolépticos, tais como a clorpromazina ou haloperidol, causa um aumento no metabolismo central da dopamina, o que se comprova pelo aumento da atividade da enzima tirosina hidroxilase, bem como das concentrações dos ácidos dihidroxifenilacético e homovanílico (Bannon et al. 1983; Carlsson & Lindqvist 1963; Coyle et al. 1985; Ericson et al 1996; Fekete e Borsy 1971; Kolenik et al. 1989; Nyback e Sedvall 1969). Assim, é possível que as alterações acima referidas, associadas à administração destas

drogas, sejam responsáveis pela diminuição da hipossensibilidade dos animais desnutridos aos seus efeitos comportamentais, o que foi observado ao se comparar os resultados do segundo dia de tratamento em relação ao primeiro dia. Por outro lado, há evidências de que os efeitos comportamentais e bioquímicos de agentes dopaminérgicos sejam influenciados pela novidade (Bardo et al. 1990). No primeiro dia de tratamento do presente estudo, os animais desnutridos apresentaram-se mais ativos no campo aberto, em comparação com os animais normais, o que pode indicar que estes animais estejam interagindo com a novidade de forma diferente dos animais normais. Outros estudos demonstraram que ratos submetidos à desnutrição em idade precoce apresentam, na idade adulta, repostas comportamentais e neuroquímicas diferentes a novas situações, quando comparados com animais normais (Mello et al. 1989; Perry et al. 1989; Rocha & Mello 1994; Venedite et al. 1988; 1990). Portanto, é possível que a hiperreatividade apresentada pelos ratos desnutridos tenha contribuído para a hipossensibilidade aos efeitos comportamentais das drogas testadas, observada neste grupo de animais. Ainda, a atividade locomotora mais intensa, observada nos animais desnutridos tratados com salina, a partir da segunda sessão, em comparação com os animais normais, pode representar uma habituação mais rápida dos animais normais ao ambiente do teste.

A hipossensibilidade dos animais desnutridos aos efeitos cataleptogênicos da clorpromazina e do haloperidol também foi verificada quando aos efeitos cataleptogênicos do SCH23390. Porém, quanto à inibição da atividade locomotora, não foi possível notar diferença entre os grupos nutricionais. Isto pode estar relacionado ao fato de que a catalepsia e a atividade locomotora sejam reguladas por diferentes vias dopaminérgicas (Hillegaart et al. 1988). A catalepsia parece estar relacionada às ações dos neurolépticos sobre o corpo estriado (Fog et al. 1970; Beninger, 1983), o qual faz parte dos núcleos da base, estando associado a sistema extrapiramidal. Demonstrou-se que microinjeções de neurolépticos produzem catalepsia apenas quando estes são injetados no corpo estriado, não ocorrendo o mesmo

quando injetados em outras áreas cerebrais (Fog et al, 1970). Já a atividade locomotora sofre influência de outras vias neurais, tais como a via mesocorticolímbica (Fink et al. 1980; Museo e Wise,1990; Beninger, 1983).

Wiggins et al.(1984) sugeriram que a hipossensibilidade de ratos desnutridos a agonistas dopaminérgicos estaria relacionada a uma redução do número de receptores. Esta hipótese não pode ser descartada, contudo outros componentes intracelulares, participantes do acoplamento e da transdução do sinal, também podem ter sido afetados pela desnutrição, o que aumenta a complexidade desta análise, uma vez que tanto fatores farmacocinéticos quanto farmacodinâmicos parecem estar envolvidos. Uma possível maneira de se elucidar esta questão seria através da administração destas drogas diretamente dentro do sistema nervoso central, ou seja, através de injeções das drogas nos ventrículos laterais do cérebro, o que eliminaria os efeitos da absorção, distribuição e eliminação das drogas, simplificando a análise.

Em resumo, pode-se concluir que ratos jovens, desnutridos, apresentam menor sensibilidade aos efeitos cataleptogênicos e inibidores da atividade locomotora da clorpromazina e do haloperidol, bem como aos efeitos cataleptogênicos do SCH23390. Estes achados estão de acordo com a maioria dos trabalhos encontrados na literatura, os quais demonstram, de uma forma geral, uma hiporresponsividade dos animais desnutridos a vários agentes de ação central (Almeida et al. 1996). O fato de haver efeitos comportamentais inicialmente similares em animais normais e desnutridos, porém uma recuperação mais rápida destes efeitos nos animais desnutridos, sugere que fatores farmacocinéticos possam estar envolvidos, resultando numa eliminação mais rápida destas drogas pelos animais desnutridos. Torna-se evidente a necessidade de se estudar a farmacocinética destas drogas em animais desnutridos, dada a grande escassez de dados relativos a este tópico (Almeida et al.1996), bem como em relação aos animais normais.

ABSTRACT

Undernutrition during critical periods of development may cause changes in the behavioral responses of rats to centrally acting drugs. In the present study, the effects of undernutrition during suckling on the behavioral responses of 21-days-old rats to chlorpromazine (0, 2.5, 5, 10 and 20 mg/kg), haloperidol (0, 0.125, 0.25, 0.5, 1 or 2 mg/kg) and SCH23390 (0, 0.3, 0.6 and 1.2mg/kg) were examined. Locomotion was assessed at 1 h 30 min, 4 h 30 min, 7 h 30 min and 10 h 30 min, and catalepsy was scored at 3 h, 6 h and 9 h after chlorpromazine or haloperidol administration. SCH23390 effects on locomotion and catalepsy were assessed at 25 min, 1 h 10 min, 1 h 55 min and 2 h 40 min after drug administration. Drugs were injected on two consecutive days. On day 1, saline-treated undernourished rats showed significant greater locomotion activity than did normal rats. The neuroleptic-induced inhibition of locomotor activity in undernourished rats was significantly less than that observed in normal rats from 4 h 30 min to 10 h 30 min (chlorpromazine) or from 7 h 30 min to 10 h 30 min (haloperidol). SCH23390 induced a similar inhibition of locomotor activity in both normal and undernourished animals. On day 2, a similar trend was observed but only in rats injected with 5 mg/kg chlorpromazine or 0.5, 1, and 2 mg/kg haloperidol. On day 1, the catalepsy scores at 3 h revealed no significant difference between nutritional groups, but at 6 h undernourished rats responded significantly less to chlorpromazine and haloperidol. SCH23390-induced catalepsy was reversed differently in undernourished animals compared to normal animals with resulting lower catalepsy scores at the 1 h 55 min and 2 h 40 min assessments for the undernourished animals on day 1. On day 2, undernourished rats were less responsive to neuroleptics than normal rats, but the effect was not so evident as observed on day 1. The present results suggest that the behavioral effects of chlorpromazine, haloperidol and SCH23390 are less persistent in undernourished rats, possibly due to differences in drug distribution and elimination, when compared to well-nourished rats.

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