Effect of fenfluramine on caloric intake and macronutrient selection in Lou/c rats during aging

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Received 6 December 2001; received in revised form 6 February 2002; accepted 21 February 2002

Abstract

Previous studies have shown a shift of preferences from carbohydrate to fat and a decrease in protein intake in self-selected Lou/c rats with advancing age. This study investigated a potential neurochemical mechanism underlying age-related modifications by evaluating the effects of fenfluramine (dl-F), a drug that enhances 5-HT release and blocks its re-uptake by presynaptic terminals, on macronutrient selection. The drug dl-F (1.5 and 3 mg/kg s.c.) induces a dose-related hypophagia with the oldest animals being the most sensitive. The main decrease is in fat consumption with minor changes in carbohydrate and protein consumptions. Young, but not old animals, compensate during the day the nocturnal intake decrease induced by dl-F. The plasma concentration of dexfenfluramine (d-F) was higher as the rats aged. The icv administrations of dl-F induced a caloric intake decrease in the oldest groups and a differential effect on protein intake between old and young rats. Metergoline induced a partial reversion of dl-F effect on food intake but this effect was not age related. These data suggest a possible implication of serotoninergic system in modifications of food behavior during aging. However, further studies are needed.

Keywords: Food intake; Macronutrients; Aging; Fenfluramine; Metergoline; Serotonin; Lou/c rat

1. Introduction

Studies performed on rats have shown modifications in caloric intake and dietary preferences as a function of age when animals were allowed to choose their diet from pure macronutrient sources (protein, fat, and carbohydrate). Carbohydrate intake has been found to decrease and fat intake to increase in growing rats [17], and protein intake was unchanged [25] or increased from weaning to maturity [21]. However, very few studies have dealt with modifications of dietary preferences during aging. The results obtained in our group showed a shift of preferences from carbohydrate to fat diet, and a decrease in protein intake in the Lou/c/jall rat with advancing age [4,34,35].

The mechanisms underlying modifications in food intake and macronutrient choices during aging are now under investigation. Many studies provided extensive evidence for the role of brain monoamines in the control of food intake, meal pattern and appetite for specific macronutrients [16,21,22]. Among the specific neurotransmitters involved in appetite regulation, serotonin (5-HT) had been shown to suppress appetite after systemic or central administrations [3]. Serotonin was also shown to have an effect on macronutrient selection [23]. These results depend on the available diet choice. If the animal could make a free choice from two isocaloric diets, high-protein or -carbohydrate diets, injection of 5-hydroxytryptamine (5-HT) into the hypothalamic paraventricular nucleus (PVN) decreased the proportion of carbohydrates [23,37]. Recent studies support a selective action to suppress fat intake when the pure macronutrients (protein, fat and carbohydrate) are offered separately to the rats [13,32,33].

Furthermore, the 5-HT system seems to be affected by aging. Goicoechea et al. [11] had shown an age-dependent decrease in 5-HT level in several brain areas. This could be linked with the observed decrease in plasmatic precursor concentration (tryptophan), tryptophan hydroxylase activity [15,26,29] and increase in MAO activity [2,29] during aging. However, a recent study shows that the age-related modifications in 5-HT concentration in the brain differs from one area to another [28]. These data suggest a complex evolution of serotoninergic system with aging.

Consequently, the involvement of the serotoninergic system in the age-related observed modifications could be...
hypothesized. Various approaches could be used to test this hypothesis; among them, pharmacological studies could give relevant information. Indeed, modification in the reactivity to an exogenous stimulation/inhibition of the serotonergic system in animals at different ages could help to understand the contribution of functional modification in the serotonergic system in the observed age-related behavioural changes. The present experiments are aimed at studying the effect of fenfluramine (dl-F), a drug that enhances 5-HT release and blocks its re-uptake by presynaptic terminals, as a function of age.

2. Materials and methods

2.1. Animals

Male Lou/c rats (Harlan, France) were used. These rats exhibit a lighter body weight with no development of obesity with age. Rats were housed in pairs in plastic cages and maintained at 22 ± 1 °C on a 12:12-h cycle (lights off at 20:00 h) with food and water available ad libitum. Before the present experiment, all rats had been allowed to adapt to the self selection procedure from the age of 4 months according to the procedure routinely used in the laboratory. Briefly, after habituation to a powered complete diet for 2 weeks, they have been allowed to choose their regimen in a self selection for 3 weeks. Nutrient choices were stabilized within this time [34].

2.2. Self-selection diet

The self-selection diet was provided as separate sources of the three pure macronutrients. The protein component (metabolizable energy 3.3 kcal/g) was composed of 93% casein (Louis-François, France). The fat component (7.88 kcal/g) contained 91% lard +2% sunflower oil, and the carbohydrate component (3.34 kcal/g) consisted of 85% corn starch (Cerestar 12018 Louis-François) and 8% commercial grade cellulose powder (U.A.R.), 1% vitamins (U.A.R. 200) and 4% salt mixture (U.A.R. 205 b).

2.3. Drugs

The drug dl-F (Sigma, France) in doses of 1.5 and 3 mg/kg body weight and 100 and 200 µg was dissolved in saline (NaCl 9%) or Metroplone (Sigma, France) at 100 and 200 nmol was dissolved in dimethyl sulfoxide (DMSO, Sigma, France).

2.4. Stereotaxic surgery

Rats were anaesthetized with ketamine chloride (80 mg/kg) and implanted with intracerebral cannulas from a flat skull position. The tip of the 25-gauge stainless steel guide cannula was aimed according to the following stereotaxic coordinates relative to bregma into the third ventricle at midline: −1.33 mm caudal and at a depth of −7.2 mm. The guide cannula was anchored to the skull with two stainless steel screws and dental cement and then closed with a 30-gauge wire obturator. Rats were allowed to recover for at least 5 days before experimental testing began.

2.5. Determination of plasma concentration

Dexfenfluramine (d-F) and norfenfluramine (NF) determinations in rat serum were performed using a previously published method [1,24]. After alkalinisation of serum samples and addition of the internal standard (d5-methamphetamine), d-F and its metabolite were extracted using diethyl ether, derivatized with heptafluorobutyrlic anhydride, then purified by successive washing with deionized water and 4% NH4 OH. Chromatographic separation was performed using a Shimadzu GC-17A gas chromatograph equipped with a split/splitless injector and a Supelco PTE 5 30 m × 0.32 i.d., 0.25-µm film thickness capillary column. Detection was carried out with a Shimadzu QP 5000 mass spectrometer detector in the electron-impact, selected ion-monitoring mode, using three mass-to-charge ratios (one for quantitation and two for confirmation) for the two analytes: m/z = 268 (240, 159) for d-F; m/z = 540 (380, 186) for NF and m/z = 258 for d5-methamphetamine. The limit of quantitation was 20 µg/l and the method was linear from this limit up to 1000 µg/l for the two analytes [24].

2.6. Experimental procedure

This study was carried out with 94 male rats divided into four groups of age: mature, middle-aged, old and senescent. In order to be able to compare our data with those of the literature, the drugs and protocol used in this study were close to that used by Smith et al. [33], doses of dl-F were doubled in our study because we used dl-F instead of d-F and it has been demonstrated that dl-F is half as effective as d-F [14].

2.6.1. Experiment 1: effect of subcutaneous administrations of dl-F (1.5 and 3 mg/kg body weight)

Three groups of mature (12 months, n = 12), middle-aged (18 months, n = 12) and old (24 months, n = 8) Lou/c rats, were used. Before the start of the experiment, self-selected rats were given saline injections for 6 days to habituation. The mean of the last 3 days of habituation was used as control.

Rats were injected as following:

- On day 1 rats were injected with dl-F 1.5 mg/kg.
- On day 2 rats were injected with saline.
- On days 3–5, rats were injected with dl-F 1.5 mg/kg.
- On days 6–8, rats were injected with saline.
The same procedure was used for the 3 mg/kg dose of dl-F (days 9–19).

Plasma concentrations of d-F (active enantiomer) and NF (active metabolite) were determined 1 h after a 3 mg/kg subcutaneous (s.c.) injection in 10 rats of each group. The blood sampling were performed in the retro-orbital sinus after a light anesthesia with halothane.

2.6.2. Experiment 2: effect of third cerebroventricular injections of dl-F (100 and 200 µg)

Four groups of mature (6 months, n = 6), old (21 months, n = 7) and senescent (27 months, n = 8 and 33 months, n = 11) rats were used. Before the start of the experiment, self-selected rats were given saline injections for 3 days to habituation. These days of habituation were used as control. Then, animals were injected with 100 µg of dl-F. The same procedure was used for the dose of 200 µg.

The injections were performed via a cannula implanted in the third ventricle near the paraventricular nucleus [33].

2.6.3. Experiment 3: effect of third cerebroventricular injection of 5-HT receptor antagonist metergoline on anorexia induced by systemic administered dl-F

Three groups of mature (8 months, n = 3) and senescent (29 months, n = 7) male Lou/c rats, were used. Before the start of the experiment, self-selected rats were given DMSO injections for 3 days to habituation. The last day of habituation was used as control, then, the rats were submitted to an icv (third ventricle) administration of metergoline 100 nmol. The same procedure was used for the dose of 200 nmol.

In the second part of the experiment, rats were submitted to an icv injection of metergoline or solvent (DMSO) 30 min after a subcutaneous injection of dl-F or saline. The rats were divided in three groups: control group (saline s.c. + DMSO icv), fenfluramine group (dl-F s.c. + DMSO icv) and fenfluramine/metergoline group (dl-F s.c. + metergoline icv).

2.7. Measurements

Body weight and macronutrient intake of all rats were recorded twice a day, at 08:00 and 18:30 h while animals were injected. To ensure minimal disturbance to the animal’s food behavior, fresh food was introduced the morning (08:00 h).

Video recordings were made during the nights of the 3rd control day and of the last subchronic injection of dl-F 1.5 mg/kg s.c. Analysis was carried on time spent to eat each macronutrient, to locomotion and to grooming for four rats of each age group.

2.8. Statistical analysis

All data are given as mean ± S.E. Effect of treatment was evaluated using analysis of variance (ANOVA) with repeated measures performed on control and experimental groups. ANOVA with repeated measures was followed by a least square mean test for an a-posteriori mean comparison (significance at P < 0.05).

3. Results

3.1. Caloric intake and macronutrient choices according to age

When we consider the regimen of animals before injections, whatever the experiment, there was an effect of age (F(6, 80) < 19.06, P < 0.0001) on diet composition expressed by the percentage of caloric intake for each macronutrient. As previously observed in Lou/c/jall rats [4,34,35], the percentage of protein in the regimen was diminished and a shift in energetic macronutrient intake, i.e. more fat and less carbohydrates was observed with increasing age (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of the regimen and caloric intake in each age-group, at the end of the self-selection procedure habituationa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial regimen (%)</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>12 Months</td>
<td>24.9 ± 2.8</td>
</tr>
<tr>
<td>24 Months</td>
<td>12.8 ± 2.5abc</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>20.6 ± 1.7</td>
</tr>
<tr>
<td>21 Months</td>
<td>14.0 ± 1.2a</td>
</tr>
<tr>
<td>27 Months</td>
<td>12.3 ± 0.8bc</td>
</tr>
<tr>
<td>33 Months</td>
<td>11.0 ± 1.0a</td>
</tr>
</tbody>
</table>

a Values are mean ± S.E. from self-selected intake in three-choice, macronutrient diet protocol by non-deprived rats during 24 h.
b Different from 12 (experiment 1) or 6 months (experiment 2), P < 0.05.
c Different from 18 (experiment 1) or 21 months (experiment 2), P < 0.05.
3.2. Experiment 1: effect of subcutaneous administrations of dl-F (1.5 and 3 mg/kg body weight)

The first administrations of saline (before dl-F 1.5 mg/kg) decreased 24 h caloric intake ($F(1,13) = 38.9, P < 0.0001$) without changing diet composition. This modification was undoubtedly due to a stress caused by the injections in spite of the habituation phase. Influence on caloric intake and regimen composition was no longer observed during the following saline injections ($F(1,13) = 0.3, NS$).

3.2.1. Effect of dl-F on caloric intake

Results show that subcutaneously injected dl-F reduced 24-h caloric intake both after acute (1.5 mg/kg, $F(2,26) = 25.1, P < 0.0001$; 3 mg/kg, $F(2,26) = 55.0, P < 0.0001$) and subchronic administrations (1.5 mg/kg, $F(3,39) = 23.1, P < 0.0001$; 3 mg/kg, $F(3,39) = 247.6, P < 0.0001$). This effect was dose-dependent for acute ($F(1,13) = 32.6, P < 0.0001$) and for subchronic administrations ($F(1,13) = 58.7, P < 0.0001$). It was age-related when the drug was acutely administered and oldest animals were the most sensitive (27% and 60% decrease of daily caloric intake in old rats versus 9% and 34% in middle aged and 9 and 28% in mature ones, with the dose of 1.5 and 3 mg/kg, respectively) (Fig. 1).

The 24h decrease was due to a strong dose-dependent decrease of food intake during the night ($F(1,13) = 678.73, P < 0.0001$). During the diurnal phase, rats were able to increase their caloric intake. A dose effect was observed ($F(1,13) = 604.8, P < 0.0001$) showing that compensatory intake was higher after the 3 mg/kg administration (acute conditions: $F(1,13) = 316.9, P < 0.0001$; subchronic conditions: $F(1,13) = 79.9, P < 0.0001$). An effect of age on daily intake was seen after acute injection of dl-F 3 mg/kg ($F(2,13) = 3.7, P < 0.05$). Oldest rats showed a less efficient compensatory intake (+6.5 ± 1.3 kcal) than younger ones (+16.0 ± 2.5 and +14.5 ± 2.6 kcal).
3 mg/kg dose). and increased whatever dose and age (24-month-old rats, respectively). The fat consumption was decreased 

\[ \times (\text{+} \pm 1) \] 

after subchronic administration. After acute injection, a weaker dose of dl-F did not modify the time spent to eat whatever the age-group. However, as the amount of food intake was significantly reduced (\(F(1, 8) = 38.2, P < 0.0005\)), efficiency in eating expressed in time taken (min) to eat 1 g of food was decreased. The results of the video recordings are presented in Table 3. During the night, subchronic administration of dl-F did not modify the time spent to eat whatever the age-group. However, as the amount of food intake was significantly reduced (\(F(1, 8) = 38.2, P < 0.0005\)), efficiency in eating expressed in time taken (min) to eat 1 g of food

**Table 2.** Effect of systemic injections of dl-F on macronutrient intake.

<table>
<thead>
<tr>
<th>Dose of dl-F</th>
<th>Age (months)</th>
<th>Caloric intake change (%)</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protein</td>
<td>Fat</td>
</tr>
<tr>
<td>1.5 mg/kg, s.c.</td>
<td>12</td>
<td>-12.4 ± 8.2(^1)</td>
<td>-11.9 ± 11.9</td>
<td>-30.8 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>-15.2 ± 4.4(^3)</td>
<td>-16.0 ± 6.2</td>
<td>4.7 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-23.1 ± 8.4(^2)</td>
<td>-30.2 ± 9.8(^2)</td>
<td>-20.3 ± 14.7</td>
</tr>
<tr>
<td>All groups</td>
<td>12–24</td>
<td>-16.1 ± 3.5(^2)</td>
<td>-18.0 ± 5.6(^2)</td>
<td>-7.4 ± 5.6</td>
</tr>
</tbody>
</table>

\(^{1}\) Values are changes in each macronutrient intakes expressed as percentage of initial caloric intakes \(\pm \text{S.E.}\).  
\(^{2}\) Significant change of intake \(P < 0.05\).  
\(^{3}\) Different from 12-month-old rats, \(P < 0.05\).

**3.2.2. Effect of dl-F on macronutrient choice**

Intake of the three macronutrients decreased after acute and subchronic dl-F administrations. This effect was dose-related, the diminution was lower at the 1.5 mg/kg dose than at the 3 mg/kg dose. At this last dose, macronutrient consumption was strongly affected. Whatever the dose, fat intake was the most diminished in old rats (interaction age \(\times\) effect \(F(2, 13) = 9.45, P < 0.005\), undoubtedly because of a higher fat intake in this group during the control period (Table 2).

During the diurnal period, compensatory intake was observed on the intake of the three macronutrients after subchronic administration. After acute injection, a weaker dose of dl-F induced an increase of protein intake only in the youngest group (interaction age \(\times\) effect \(F(2, 13) = 3.99, P < 0.05\) \((+1.7 \pm 0.7 \text{kcal for 12 months versus } -0.2 \pm 0.3 \text{ and } -0.1 \pm 0.5 \text{kcal for 18 and 24 months}), whereas a higher dose induced an increase in carbohydrate intake \((F(1, 13) = 197.3, P < 0.0001)\) especially for the mature group (interaction age \(\times\) effect \(F(2, 13) = 6.0, P < 0.02\) \((-8.8 \pm 0.7, +6.1 \pm 0.9 \text{ and } +4.4 \pm 0.7 \text{kcal for 12, 18 and 24-month-old rats, respectively})

The fat consumption was increased whatever dose and age (+9.7 ± 3.1, +8.0 ± 2.4 and +14.2 ± 2.2 kcal for 12-, 18- and 24-month-old rats at 3 mg/kg dose).

**3.2.3. Effect of dl-F on body weight**

dl-F administration induced a body weight loss \((F(1, 28) < 22.8, P < 0.0007\) for acute injection and \(F(3, 84) < 29.5, P < 0.0001\) for subchronic one). In subchronic conditions, an interaction age \(\times\) effect was seen \((F(12, 168) = 1.68, P < 0.002\) and \(F(12, 168) = 2.5, P < 0.005\) for 1.5 and 3 mg/kg, respectively), for example, the mean loss for the 3 days of dl-F injections (3 mg/kg body weight) was \(-1.8 \pm 0.6, -6.2 \pm 2.6 \text{ and } -4.3 \pm 0.4 \text{ g for 12-, 18- and 24-month-old rats, respectively}.

**3.2.4. Plasma concentration of dl-F and NF**

One hour after injection of 3 mg/kg of dl-F, the plasma concentration of dl-F was dependent on age and was higher in older rats \((F(2, 25) = 5.26, P < 0.02)\). The plasma rate of the metabolite (NF) was not modified by age \((F(2, 25) = 1.37, \text{ NS})\) (Fig. 2).
was decreased for all groups \((F(1,8) = 11.2, P = 0.01)\). Moreover, it induced changes in activity. Time spent in locomotion was reduced \((F(1,8) = 56.7, P < 0.001)\) and this effect was seen only in older groups (interaction age \(\times\) treatment \(F(2,8) = 14.4, P = 0.002\)). Time spent in grooming increased in all groups \((F(1,8) = 5.5, P < 0.05)\).

### 3.3. Experiment 2: effect of third cerebroventricular injection of dl-F (100 and 200 \(\mu\)g)

The first icv administrations of vehicle (before dl-F 100 \(\mu\)g) induced a drop in 24h caloric intake \((F(1,37) = 35.01, P < 0.0001)\) and a change in diet composition \((F(2,74) = 20.2, P < 0.0001)\). It decreased the proportion of protein and fat intakes and increased carbohydrate intake. This effect was more important in the youngest group (interaction age \(\times\) effect \(F(6,74) = 2.35, P < 0.04)\).

The second icv injection of vehicle (before dl-F 200 \(\mu\)g) had a weaker effect \((F(1,28) = 10.29, P < 0.0004)\). Compared to non-injected conditions, caloric intake remained stable but the regimen was lightly modified \((F(2,56) = 10.38, P < 0.0001)\), the proportion of fat decreased and carbohydrates increased.

#### 3.3.1. Effect of dl-F on caloric intake

There were no significant difference on caloric intake after the dl-F dose of 100 \(\mu\)g and vehicle treatment (results not shown).

A 200-\(\mu\)g dose of the drug induced a decrease in 24-h caloric intake \((F(1,28) = 38.96, P < 0.0001)\) due to a diminution of intake during the night \((F(1,28) = 46.36, P < 0.0001)\) as in systemic treatment. The 24-h decrease was also dependent on age (interaction age \(\times\) effect \(F(3,28) = 4.13, P < 0.02)\) (Fig. 3) due to an interaction age \(\times\) effect \((F(3,28) = 3.78, P < 0.03)\) found in diurnal intake. Young rats increased (+4.4 \pm 2.9 kcal) their caloric intake whereas the oldest ones decreased it \((-3.1 \pm 2.1, -4.2 \pm 1.8, -5.1 \pm 1.7 kcal\) respectively for 21-, 27- and 33-month-old rats).

#### 3.3.2. Effect of dl-F on macronutrient choice

The administration of weaker dose of dl-F had no effect on macronutrient choices (Fig. 4).

Higher dose (200\(\mu\)g) significantly decreased 24-h fat intake by 39% on average \((F(1,28) = 48.99, P < 0.0001)\). Protein intake increased in the 6-month-old rats and decreased in other age groups (interaction age \(\times\) effect \(F(3,28) = 4.7, P < 0.01)\). The percentage of proteins in
Fig. 4. Effect of icv administration of dl-F (200 μg) on protein, fat and carbohydrate intakes in 6-, 21-, 27- and 33-month-old rats, measured during night and day. C, mean value for the 3 days of saline injection; F, dl-F. ∗P<0.05 compared with saline injection (a-posteriori test).

the diet reached 23.2 ± 6.1% versus 15.7 ± 2.3% in saline conditions.

When intake was analyzed according to nocturnal or diurnal period, a reduction of fat intake was seen whatever the period (night: \( F(1, 28) = 60.0, P < 0.0001 \) and day: \( F(1, 28) = 10.43, P < 0.004 \)).

During the night, carbohydrate intake decreased (\( F(1, 28) = 4.57, P < 0.05 \)) and the effect observed on proteins depend on age (\( F(3, 28) = 3.72, P < 0.03 \)); protein intake increased for the young rats and decreased for the oldest ones. During the diurnal period, an interaction age \( \times \) effect was seen for carbohydrate intake (\( F(3, 28) = 3.06, P < 0.05 \)): young animals (6 months) increased carbohydrate intake, whereas it remained at the same level in older groups.

3.3.3. Effect of dl-F on body weight

A body weight loss was observed (\( F(1, 28) = 35.51, P < 0.0001 \)) after injection of the dl-F dose of 200 μg, it was not age-dependent. All rats lost 6.9 ± 1.4 g on average.

3.4. Experiment 3: effect of third cerebroventricular injections of 5-HT receptor antagonist metergoline on anorexia induced by systemic administered dl-F

3.4.1. Effect of icv administrations of metergoline

The icv administrations of metergoline vehicle (DMSO) did not induce modification in 24-h caloric intake (\( F(1, 14) < 1.95, \text{NS} \)) or in diet composition (\( F(2, 28) < 0.12, \text{NS} \)).

No modification in 24 h, nocturnal and diurnal caloric intakes or diet composition (expressed as percentage of each macronutrient) was observed when metergoline (100 nmol) was centrally administered (\( F(1, 18) < 4.4, \text{NS} \) for caloric intake and \( F(2, 34) < 0.7, \text{NS} \) for diet composition) (results not shown).

When a metergoline dose of 200 nmol was injected in the third ventricle, caloric intake and macronutrient choices were not modified, whatever the age group.
3.4.2. Influence of icv administrations of metergoline on effects induced by systemic administered dl-F

As observed in the first experiment, dl-F (3 mg/kg s.c.) induced an obvious decrease in caloric intake measured 24 h after injection ($F(1, 7) = 49.79, P = 0.0002$) due to a strong decrease during the nocturnal phase ($F(1, 7) = 42.39, P = 0.0003$). Mainly fat intake decreased ($F(1, 7) = 28.98, P < 0.001$) but also carbohydrate ($F(1, 7) = 24.71, P < 0.02$) and protein ($F(1, 7) = 12.68, P < 0.01$) intakes decreased slightly (Fig. 5).

The co-administration of metergoline (100 nmol, icv) led to a partial reversion of the anorexigenic effect of dl-F ($F(1, 7) = 9.15, P < 0.02$) linked to a rise of intake of the three macronutrients. These effects were not age-related ($F(1, 7) < 0.5, NS$), whereas they tended to be more marked in the oldest group.

4. Discussion

Systemic and central administrations of dl-F induced a dose-dependent hypophagia. These experimental data confirm the potent anorexigenic effect of fenfluramine widely described in the literature (see references in the review of Noach [27]). Fenfluramine is known to induce a release of serotonin and an inhibition of its re-uptake at the synaptic level. Systemically administered fenfluramine, along with its active metabolite norfenfluramine, readily cross the blood-brain barrier. From a neurochemical point of view, the effect of dl-F may be accounted for by its central action to increase serotonergic transmission in the hypothalamus [30] or by a direct action on 5-HT post synaptic receptors [10,36]. According to the most largely accepted theory [20], intake regulation would be primarily controlled by the balance between serotonergic and noradrenergic hypothalamic systems. dl-F would lead to a shift of this balance in favor of the serotonergic system that causes an earlier induction of satiety and thus a decrease in food intake. Nevertheless, a lot of studies highlight the complexity of the interaction between serotonin and feeding behavior (see review of Halford and Blundel [12]). Few studies have dealt with the influence of age on this anorexia. Our study shows that after acute systemic injection, an age-dependent decrease in caloric intake was observed. The oldest animals proved to be the most sensitive to drug injections. These differences observed in response to systemic administration of fenfluramine could be interpreted in three ways: (i) modifications in the dynamics of serotonergic regulation during aging, (ii) kinetic modifications, and finally (iii) modifications in the ability to compensate after the injection induced anorexia. This last assumption is confirmed by the comparison of data concerning food intake during the night and during the day. This analysis indicates that during the day, young animals compensate for the nocturnal decrease in food intake induced by the administration of dl-F, but oldest animals do not show this compensatory behavior. Such observations are in agreement with those from work concerning the endogenous opioid system [5]. Nevertheless, differences in the ability of compensation along diurnal period are not sufficient to explain all the observed differences. The age-related differences can also be interpreted as being related to pharmacokinetic differences. Physiological changes associated with aging, such as the decrease in liver or renal function, could induce differences in plasmatic concentration of fenfluramine in older rats and also explain an increased effect of the drug. Indeed, we showed that 1 h after the injection of a dose of 3 mg/kg of dl-F, the plasmatic rates of d-F (effective isomer) was higher as the animals were older. As it is well known for many drugs, aging leads to a reduction in the metabolism and a decrease in the clearance of dl-F [8]. If these modifications are likely to explain, at least partly, the effects observed after systemic administration of dl-F, it must be noted that other mechanisms are involved. Indeed, after central injection of the drug, which allows to short-circuit the pharmacokinetic problems, age differences were observed. Modifications in the central serotonergic system dynamic cannot be excluded and the present data could result from functional changes...
in the brain 5-HT system inducing a higher sensitivity to the physiological effects of dl-F.

It should be noted that some studies highlighted other effects of dl-F, particularly at the peripheral level [6]. Boschman et al. [7] have shown that dl-F induced alterations in peripheral energy metabolism. Some differences observed after systemic injections could also be due to modifications in peripheral metabolism across aging.

These results are enhanced by data related to the administration of metergoline, a broad spectrum antagonist of the 5-HT receptors. If differences related to aging exist in the tonic regulation of food intake by the serotonergic system, the effect of metergoline must be modified by age. Our results did not agree with this hypothesis because when the metergoline is administered alone, no effect is observed whatever the age group considered. This lack of effect will have to be confirmed since the study of Smith et al. [33] performed on 9-month-old Wistar rats shows that the administration of the same dose of metergoline as we used, led to an increase in the consumption of fat (and by consequence of the caloric intake measured during 2h). Many authors insist on the complexity of the interactions and recommend the use of more selective antagonists than metergoline [31]. Lastly, results obtained by administration of metergoline in the third ventricle 30 min after subcutaneous injection of dl-F confirm the data of the literature [27]. The nocturnal intake which was decreased by 80% after dl-F injection, is brought back to 53% of its initial value when the receptors were blocked by metergoline. The reversion of the anorexigenic effect of dl-F is thus partial. In addition, no influence of age was shown on the effect of metergoline.

Regarding diet composition, the previously described modifications in feeding patterns with advancing age in Long/ejall rats are confirmed herein. When they become older, rats select more fat and less carbohydrates, protein intake also decreases.

After systemic administration of fenfluramine, decrease in caloric intake was due to a drop in the three macronutrient intakes with fat consumption decreasing most. This result agrees with data that support a selective action either of systemically administered serotonin or of its receptor agonists to suppress fat intake both in animal studies designed to allow a concurrent evaluation of the consumption of individual fat, carbohydrate, and protein diets [13,18] and in human studies where macronutrient content of meals was assessed directly [19]. However, it has been observed that dietary fat but not carbohydrate intake was selectively suppressed in response to systemic treatment with dl-F [32].

One of the most interesting result is the differential effect induced on protein intake by icv administration of dl-F. The increase in protein intake observed in young rats could suggest that a stimulation of the serotonin system led to an increase in specific protein consumption. As dl-F is not able to induce an increase in protein intake in old animals, it could be hypothesized that the decrease in protein intake observed throughout life [34] might be linked to a loss of sensitivity to serotonin. This result has not yet been reported in the literature, however, differences in experimental procedures could also account for this discrepancy. Compensatory intake was dose- and age-dependent. After systemic administration, fat intake increased in all age groups, whereas in the youngest animals an increased protein intake in response to the weak dose and an increased carbohydrate intake in response to the high dose of dl-F were observed. When central administrations were used, no compensatory intake was seen in the oldest groups, whereas in the young one, compensation appears to be due to an increase in carbohydrate intake.

Our results show the well-described hypophagia induced by dl-F. This hypophagia is linked to a decrease in energetic nutrient (fat) intake mainly in old animals. Our results also show the influence of kinetic parameters and compensatory behavior in the long-term age-related effect of dl-F. The differential effect observed in protein intake after icv injection indicates that serotonergic system activity could play a role in the decrease of this macronutrient intake observed in old rats. However, further studies are needed including using short-term analysis of the serotonin effect and more specific ligands of serotonergic receptors, particularly 5-HT2C, that are involved in appetite regulation [36]. Moreover, it will be useful to obtain information about neurotransmitter levels in hypothalamic nuclei during aging, this study being in progress in our laboratory. On the other hand, there is a paucity of information regarding the effectiveness of anorectic medications in the treatment of obesity in the elderly [9]. Our experimental data may contribute to a better understanding of the physiological basis of the effect of fenfluramine in the elderly.

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