Antidepressants are prescribed not only for depression but also for a wide range of mental disorders (Baldessarini, 2001). Although they have clinical advantages, the current armamentarium of antidepressants presents an unacceptable lack of efficacy (Gumnick and Nemeroff, 2000). An important limitation in designing better antidepressants is that the mechanism of action responsible for their therapeutic effect is unknown. Although the pharmacodynamics of these drugs at molecular, cellular and system level has been investigated (e.g., Palucha and Pilc, 2002; Shelton, 2000; Shilling and Kelsoe, 2002), at cognitive level studies are less common. The purpose of the present study, and others carried out or being prepared in our laboratory, is to evaluate the effects of amitriptyline on animal cognition to relate them to some characteristics of the therapeutic effects of this and other antidepressants.

It has been suggested that the forced swimming test (FST) shares some characteristics with memory tests, such as the exploration of objects (Ennaceur and Delacour, 1988), and the exploratory behavior in a photo-cell activity cage (Platel and Porsolt, 1982), which are used to determine the effect of drugs on memory.

In this context, it is understood that in the first session of FST the animal should learn to remain immobile, and the second session would be a test of retention of what was learned in the first one (de Pablo, Parra, Segovia and Guillamón, 1989; Martos, Vinader-Caerols, Monleón, Arenas and Parra, 1999; Parra, Vinader-Caerols, Monleón and Simón, 1999).

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In Experiment 1, the effect of the administration of the antidepressant amitriptyline (30 mg/kg) for 21 days on the acquisition and consolidation of the inhibitory avoidance task was studied in male and female mice. In Experiment 2, it was evaluated whether amitriptyline administered after the consolidation of this task would block the memory retrieval. Anxiety and spontaneous activity in the elevated plus maze were also assessed. When amitriptyline was given before the training phase of inhibitory avoidance it blocked learning in males and there was a tendency in the same direction in females. However, the drug administered between training and test phases did not affect conditioning. These effects of amitriptyline seem to be independent of its actions on anxiety and locomotor activity. It may be that the effects observed are related to the therapeutic effects of antidepressants.

La amitriptilina administrada después de la consolidación de la evitación inhibitoria no afecta a la recuperación de la memoria. Se estudió el efecto de la administración, durante 21 días, del antidepresivo amitriptilina (30 mg/kg) sobre la adquisición y consolidación de la memoria de una tarea de evitación inhibitoria en ratones machos y hembras (Experimento 1). También se estudió si la amitriptilina, administrada una vez finalizado el periodo de consolidación, interfería con su recuperación (Experimento 2). En los animales de este experimento se evaluaron la ansiedad y la actividad espontánea en el laberinto en cruz elevado. La amitriptilina, cuando se administró antes de la adquisición, impidió el aprendizaje en los machos y mostró una tendencia en el mismo sentido en las hembras; cuando se administró entre la adquisición y la retención no produjo deterioro. Estos efectos parecen independientes de una acción inespecífica del fármaco sobre la ansiedad o la actividad. Se sugiere que los efectos observados pueden tener relación con los efectos terapéuticos de los antidepresivos.
whereas the other side was black and always remained dark. The Panlux Electronic2 photometer of GOSSEN, Nürnberg, Germany), white (light intensity of 290 lx at floor level, measured with the V, 10 W) in the ceiling of the starting side which was painted in white (light intensity of 290 lx at floor level, measured with the Panlux Electronic2 photometer of GOSSEN, Nürnberg, Germany), whereas the other side was black and always remained dark. The floor was made of 48 stainless steel bars of 0.7 mm in diameter and 8 mm apart.

The elevated plus-maze used in Experiment 2 (Cibertec, S.A., Madrid, Spain) was made up of two open and two closed arms (30 × 5 cm² and 30 × 15 × 5 cm², respectively) extending from a common central square (5 × 5 cm²) and elevated 50 cm above floor level on five pedestals. The maze floor was made of black Plexiglas; the open arms had no protective edge while the walls of the closed arms were made from clear Plexiglas with the external sides covered with black paper. The illumination in the experimental room consisted of four neon tubes fixed to the ceiling (light intensity of 110 lx at 50 cm above floor level). The elevated plus-maze task was recorded with a video camera (SONY Handycam CCD-TR401E, Tokyo, Japan).

**Experimental Procedures**

In Experiment 1, mice were randomly assigned by sex to one of two groups (N= 12 - 14), which received saline solution (S) or 30 mg/kg amitriptyline (A) for 21 days. The inhibitory avoidance task was carried out 24 hours after the last injection. Each mouse was individually introduced into the illuminated side of the avoidance box and permitted to explore it for an adaptation period of 90 seconds. The door between the compartments remained closed during this period. The door was then removed and the mouse could stay in the light side for a maximum of 300 seconds. If it did not enter the dark compartment in this time it was discarded, but if it entered, an inescapable footshock of 0.7mA was delivered for 5 seconds and it was immediately returned to its home cage. The inhibitory avoidance test was carried out 24 hours later, using the same procedure except that no shock was delivered. In both sessions, the latencies of crossing were measured in 1/10 seconds. The measure of the inhibitory avoidance was obtained by comparing the performance in the test session with that of the training session.

In Experiment 2, animals were randomly assigned by sex to one of two groups (saline, N= 13 - 16; amitriptyline, N= 8 - 10) using the same denominations as in Experiment 1. They were also subjected to the same treatments as in the previous experiment except that the period between sessions was 22 days and the drug administration began 24 hours after the training session and finished 24 hours before the test session. Immediately after this last phase, the exploration of each animal in the elevated plus-maze was recorded for 5 minutes. Subsequently, the number of entries onto open and closed arms (arm entry is defined as all four paws entering an arm) was scored by a trained observer unaware of the treatment applied. This provided two independent measures of anxiety, i.e. the percentage of time spent in the open arms, and the percentage of open arm entries ([open/open + closed] X 100), as well as a measure of activity, i.e. the number of closed arm entries. The rationale to select these measures is found in File (2001), Lister (1987) and Rodgers and Johnson (1995).

**Data analysis**

As data from the inhibitory avoidance task did not fulfill the criteria for normality and homogeneity, they were subjected to non-parametric Kruskal-Wallis analysis followed by Mann-Whitney U-tests. The Wilcoxon matched pairs test was carried out to compare training versus test latencies in each group. Mann-Whitney U-tests were used to compare the performance of independent groups. The elevated plus-maze behaviors were
analyzed separately with ANOVA, with Sex and Treatment as factors, and Newman-Keuls-tests were used for post-hoc analyses. All analyses were performed with the Statistica software package, version 5.5 for Windows (StatSoft, Inc., 2000).

Results

Experiment 1: Chronic pre-training treatment

Saline males showed inhibitory avoidance, i.e. test latencies higher than training latencies (T= 4.00; p<0.01), but this was not found in the males treated with amitriptyline (T= 39.00; p>0.05). Both saline and amitriptyline-treated females presented inhibitory avoidance (T= 5.00; p<0.01; and T= 10.00; p<0.03, respectively).

The treatment did not produce significant differences in the performance of animals, although a tendency in the performance of females in the test session was observed, where the drug-treated mice showed shorter latencies than controls (U= 41.00; p= 0.07). Neither were significant differences found when comparing males and females of the same drug condition (see figure 1).

Experiment 2: Chronic post-training treatment

All groups increased their test latencies in comparison with their training latencies: males S (T= 1.00; p<0.001), males A (T= 1.00; p<0.02), females S (T= 6.00; p<0.01), and females A (T= 2.00; p<0.01). No comparison with Treatment or Sex involved was statistically significant (see figure 2).

Figure 1. Effects of chronic administration of amitriptyline (30 mg/kg) before the training phase of an inhibitory avoidance task. Values are expressed as medians (± interquartile range). S: saline, A: amitriptyline. *p<0.03, **p<0.01 vs. training

Figure 2. Effects of chronic administration of amitriptyline (30 mg/kg) between training and test phases of an inhibitory avoidance task. Values are expressed as medians (± interquartile range). S: saline, A: amitriptyline. *p<0.02, **p<0.01, ***p<0.001 vs. training
In the elevated plus-maze, all female groups remained a lower percentage of time than males on the open arms \((F(1, 42) = 5.37; p<0.02)\), and neither the factor Treatment nor Sex X Treatment interaction were statistically significant \((F(1, 42) = 0.26; p>0.05, \text{ and } F(1, 42) = 1.68; p>0.05, \text{ respectively})\). The females showed a tendency to enter the open arms less than the males \((F(1, 42) = 3.60; p = 0.06)\), and again neither the factor Treatment nor Sex X Treatment interaction were statistically significant \((F(1, 42) = 0.27; p>0.05)\). When the number of entries in the closed arms were analyzed, factors did not present statistically significant differences either \([\text{Sex: } F(1, 42) = 0.13; p>0.05; \text{ Treatment: } F(1, 42) = 0.42; p>0.05; \text{ and } \text{Sex X Treatment: } F(1, 42) = 0.28; p>0.05]\\). When extrapolating results obtained in mice to humans, not only the existence of sex differences in mice in the hepatic enzymes which metabolize the drugs, specifically, the enzymatic activity is 40-100% higher in females than in males (Shapiro, Agrawal and Pampori, 1995), which would give rise to a poorer availability of the drug in the central nervous system of the females in comparison to the males. Whatever the case, our results emphasize the importance of including females in animal studies, as well as keeping in mind the factor of gender when personalizing clinical pharmacological treatment, especially since in humans, gender differences have also been described in the pharmacokinetics of many psychoactive drugs, among them antidepressants (Frackiewicz, Sramek and Cutler, 2000). However, when extrapolating results obtained in mice to humans, not only the sex differences must be taken into account but also the pharmacokinetic differences between species (Lin, 1995).

Sex differences in the behavioral effect of drugs must be analyzed taking into account that there may be a difference in the behavior of control animals, as is the case sometimes found in inhibitory avoidance (e.g., Monléon et al, 2002), and continuously in the Morris water maze (e.g., Cimadevilla, Conejo, Miranda and Arias, 2004; Vinader-Caerols et al, 2002), although in the present study there were no sex differences in control animals.

There was no drug effect on performance in the plus maze, which leads one to believe that after 21 days of administration of amitriptyline, its effects on learning observed in Experiment 1 are not influenced by its effects on anxiety or spontaneous locomotor activity. Parra et al (2002) found that there were no acute effects of amitriptyline on anxiety but there was a dose-dependant reduction in activity. In the present study, the absence of effects on activity could be explained by the possible tolerance developed after chronic administration. Furthermore, the current results replicate those obtained with the same drug and dose, as well as the same apparatus, by Everss et al (2005).

The absence of tolerance in the effect of amitriptyline on inhibitory avoidance is interesting in the search for the mechanism of therapeutic action of antidepressants. This action does not present tolerance, while the side effects tend to disappear or diminish their intensity (Badissarini, 2001). The animal models of the action of antidepressants in which tolerance is observed should be avoided.

In summary, chronic administration of amitriptyline has a deteriorating effect on inhibitory avoidance in male mice, while the effect is slighter in females. Amitriptyline exerts its effect when administered before the acquisition and consolidation of the memory but not when these have concluded. It does not present tolerance and it seems to be independent of the unspecified effects on anxiety and activity.

Acknowledgements

The authors wish to thank Arancha Ferrer Añó for her help in the experiments. The investigation has been carried out with the financial support of (a) the «Generalitat Valenciana», Valencia,
Spain (Ayuda: GV97-SH-22-89) and (b) jointly, el Ministerio de Educación y Ciencia (Spain), and the European Union, through the funds FEDER (Ayuda: BSO2003-07163). Estrella Everss received a grant from «Generalitat Valenciana» from 1999 to 2003. We also wish to thank Ms Miriam Phillips for the English translation of the manuscript.

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