Psicothema 2009. Vol. 21, nº 4, pp. 528-530
www.psicothema.com

ISSN 0214 - 9915 CODEN PSOTEG

The effect of amitriptyline on inhibitory avoidance in mice is dose-dependent

Andrés Parra, Concepción Vinader-Caerols, Aránzazu Ferrer-Añó, Adoración Urquiza and Santiago Monleón
Universidad de Valencia

The purpose of the present work was to study the dose-effect relationship of the antidepressant amitriptyline on inhibitory avoidance in male and female mice. Subjects received physiological saline or 2.5, 5, 10 or 20 mg/kg of amitriptyline hydrochloride 30 min before the training phase, and were subjected to the test phase 24 h later. Results showed a clear impairing effect of amitriptyline on inhibitory avoidance in both male and female mice, and that the effect is dose-dependent.

El efecto de la amitriptilina sobre la evitación inhibitoria en ratones es dependiente de dosis. El propósito del presente trabajo fue estudiar la relación dosis-efecto del antidepresivo amitriptilina sobre la evitación inhibitoria en ratones machos y hembras. Los sujetos recibieron suero salino o 2.5, 5, 10 ó 20 mg/kg de clorhidrato de amitriptilina 30 min antes de la fase de entrenamiento, y fueron sometidos a la fase de test 24 h más tarde. Los resultados mostraron un claro efecto deteriorante de la amitriptilina sobre la evitación inhibitoria tanto en los ratones machos como en las hembras, y que el efecto es dependiente de dosis.

It is well accepted that anticholinergic drugs impair memory (e.g., Azmi, Norman, Spicer, & Bennett, 2006). When this effect is found, a monotonic dose-response effect is expected, with some minimum dose below which no effect is observed and some maximum dose above which no further increases in impairment are produced. The dose-response relationship can be better depicted as an inverted U in some cases. In studies carried out in our laboratory dealing with the impairing effects of the antidepressant amitriptyline, the most anticholinergic drug among antidepressants (Frazer, 1997), the dose-response effect has not been found (Everss, Arenas, Vinader-Caerols, Monleón, & Parra, 2005; Parra, Everss, Monleón, Vinader-Caerols, & Arenas, 2002). These studies involved inhibitory avoidance (frequently known as passive avoidance), with one learning and one test trial that allows choosing specific moments for injection in order to affect specific memory processes like acquisition, consolidation or retrieval (Gold, 1986; Parra, Everss, Arenas, Vinader-Caerols, & Monleón, 2006). In behavioural terms, inhibitory avoidance is understood as the increase in the crossing latency from the illuminated side to the dark side when the animal is introduced for a second time into the apparatus. In this procedure, both great individual differences (each subject contributes to the mean of the group with only one score, which is obtained in only one trial) and an easily reachable ceiling effect (i.e., no crossing) could contribute to the absence of dose-response effect.

Fecha recepción: 18-2-09 • Fecha aceptación: 20-5-09
Correspondencia: Andrés Parra
Facultad de Psicología
Universidad de Valencia
46010 Valencia (Spain)
E-mail: andres.parra@uv.es

In the present work, the dose-response relationship of the effect of amitriptyline on step-through inhibitory avoidance was specifically aimed. In order to increase the probabilities of reaching the expected dose-response effect, the number of subjects per group and the range of doses were increased as compared with previous studies. The results will be helpful in designing future experiments in which drugs that supposedly enhance or interfere with the effect of amitriptyline on inhibitory avoidance in mice are administered.

Method

Animals

Subjects were 115 male and 119 female CD1 mice of 42 days of age obtained from CRIFFA (Lyon, France). Animals were housed in groups of 4 or 5 in standard translucent plastic cages of 27 × 27 × 15 cm^3 (Panlab S.L., Barcelona, Spain), in a temperature-controlled room (21 ± 2 ºC), under a reversed light/dark cycle (lights off: 07:30h-19:30h, local time), with food and water available ad libitum. Each mouse was tested only once. The tests were always carried out during the dark phase of the light/dark cycle, and took place after 7-10 days of acclimatization to the animal house. The experimental protocol and the use of animals were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Spanish Real Decreto 1201/2005.

Drugs

Amitriptyline hydrochloride (Sigma-Aldrich Química, Madrid, Spain) was dissolved in a saline solution (0.9% NaCl) to obtain the doses 2.5, 5, 10 and 20 mg/kg. These doses were chosen because,

according to earlier published studies, they do not produce sedative effects (Monleón, Vinader-Caerols, Arenas & Parra, 2008). All injections were intraperitoneally administered at a volume of 0.01 ml/g body weight.

Apparatus

A step-through inhibitory avoidance apparatus for mice (Ugo Basile, Comerio-Varese, Italy) was employed. The cage, made of Perspex sheets, was divided into two sections (both height 15 cm, width 9.5 cm, length 16.5 cm). The chambers were separated, widthwise, by a flat-box partition, with an automatically-operated sliding door at floor level. A light (24 V, 10 W, light intensity of 290 lux at floor level, measured with the Panlux Electronic2 photometer of GOSSEN, Nürnberg, Germany) was left on at all times in the ceiling of the starting side, while the other side remained in darkness. The starting side was white and the other side was black. The floor consisted of stainless steel bars, 0.7 mm in diameter and 8 mm apart.

Experimental procedures

Mice were randomly distributed into five groups for each sex (n= 20-24) and received a single injection of saline or amitriptyline (2.5, 5, 10, or 20 mg/kg) 30 min before the training session of the avoidance procedure. Training and testing began with a 90-s adaptation period in the safe chamber before the door to the other chamber was opened. During training, animals received a 5 s 0.3 mA shock when they crossed from the safe chamber into the shock chamber. During the test, mice were placed once more in the safe side of the apparatus and the procedure used in the training phase was repeated, without the shock. Latencies of step-through to the shock chamber were recorded in both phases. Crossing latencies longer than 300 s in the training phase resulted in the animal being discarded and in the test phase the trial being terminated and a latency of 300 s recorded. The training test interval was of twenty-four hours.

Data analysis

The inhibitory avoidance data were transformed into proportion (p= x/300) values and then to arc sin (arc sin $\sqrt{p}$) values according to Snedecor and Cochran (1980). Variance for training and test were analysed separately. Newman-Keuls tests were used for post hoc comparisons. Training and test sessions within the same group were compared using the Student's t test for dependent samples. All analyses were performed using the «Statistica» version 5.5 for Windows software package (StatSoft, 2000).

Results

In the training phase, Dose was statistically significant [$F(4,224)= 2.63$, $p<0.05$]. Newman-Keuls post hoc tests showed that amitriptyline at dose of 10 mg/kg increased latencies but not at lower or higher doses (see Fig. 1). Also in this phase, neither Sex nor the interaction Sex × Dose were statistically significant [$F(1,224)= 01.67$, $p>0.05$; and $F(4,224)= 1.54$, $p>0.05$; respectively].

In the test phase, Sex showed females presenting a tendency for longer latencies than males [$F(1,224)= 3.37$, $p= 0.07$]; Dose was statistically significant [$F(4,224)= 22.56$, $p<0.0001$], where the post hoc analysis showed that there were not statistically significant differences between saline and 2.5 mg/kg dose, nor among 5, 10, and 20 mg/kg doses, and the differences were significant between saline or 2.5 mg/kg dose and any other dose. The interaction Sex × Dose was also statistically significant [$F(4,224)= 2.84$, $p<0.03$]. The post hoc analysis of the interaction showed that (a) in males, the differences between saline or the 2.5 mg/kg dose and both 10 and 20 mg/kg were statistically significant, while the dose of 5 mg/kg was not statistically different from any of the other treatments; and in females, the differences between saline and the 2.5 mg/kg dose, or among the 5, 10, and 20 mg/kg doses were not statistically significant, while the differences between saline or the 2.5 mg/kg dose and all other doses were significant, and (b) that the differences between groups of male and female animals receiving the same drug treatment were statistically significant only at the 2.5 mg/kg dose (see Fig. 2).

Training and test comparisons showed that the test latencies were higher than the training latencies in saline and 2.5 mg/kg groups of males and females ($p<0.01$), and that this comparison was not statistically significant in the remaining doses ($p>0.05$), unless females receiving 20 mg/kg that showed shorter latencies in the test than in the training phase ($p<0.05$).

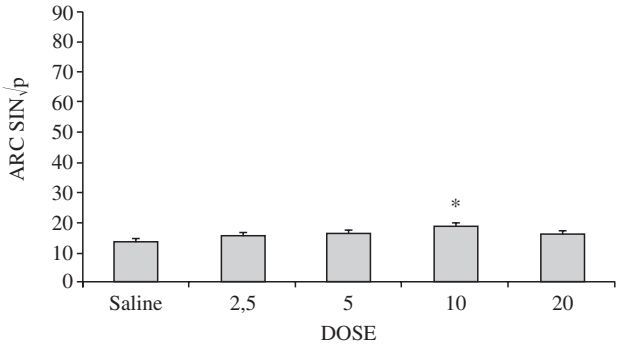

Figure 1. *Effect of pre-training administration of saline or amitriptyline (2.5, 5, 10 or 20 mg/kg) on step-through latencies in the training phase of an inhibitory avoidance task. Values are expressed as means (+SEM) of square root of proportions (p = x/300) transformed to arc sin. *p<0.05 vs Saline*

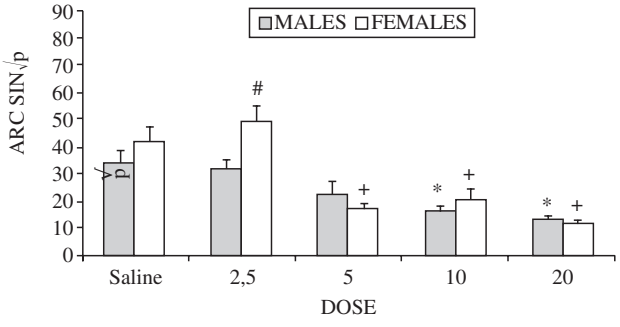

Figure 2. *Effect of pre-training administration of saline or amitriptyline (2.5, 5, 10 or 20 mg/kg) on step-through latencies in the test phase of an inhibitory avoidance task. Values are expressed as in Fig. 1. Note that, in males, 5 mg/kg was not statistically different from any of the other treatments. *p<0.05 vs Saline or 2.5 male groups; +p<0.05 vs Saline or 2,5 female groups; #p<0.05 vs males of the same drug condition*

Discussion

The present results showed a clear impairing effect of amitriptyline on inhibitory avoidance in both male and female mice, and that the effect is dose-dependent. The 2.5 mg/kg dose had no effect in either sex; 5 mg/kg had a non significant effect in males and significant in females; 10 and 20 mg/kg produced a similar and significant effect in males and females. In a previous study, 7.5, 15 and 30 mg/kg were post-training administered, and the effect was not dose-dependent (Parra et al., 2002). In the light of the present results, the mentioned study used too high doses to observe increases in the effect from the lowest to highest doses. In the training phase the drug increased the crossing latency at 10 but not at 20 mg/kg, which is an inverted-U effect. This seems another example of that the sedative effect of amitriptyline is not well correlated with an increase in response latency in inhibitory avoidance (Bammer, 1982).

Variations between the sexes in the test phase with respect to the dose of 2.5 mg/kg, by which females exhibited more avoidance than males, is an example of the sex differences found in some experiments. The presence of differences between sexes is not general, but when reported, inhibitory avoidance is consistently more pronounced in females than in males (Arenas et al., 2006). These differences are more frequent in control groups than in treated ones. The dose of 2.5 mg/kg was the lowest dose employed in the experiment, and is considered to have effects that are indistinguishable from those of saline. At present, we have no explanation for the lack of consistency in this sex difference between experiments or even within the same experiment.

Some considerations can be derived from the present results in order to better design future experiments. The lower dose, 2.5 mg/kg, seems suitable for combinations with drugs that supposedly enhance the effect of amitriptyline on inhibitory avoidance, due to this dose has no effect on behaviour by itself. Literature also shows that lower doses than 4 mg/kg of amitriptyline have no significant effects on memory in animals (for a review see Monleón et al., 2008). The 10 mg/kg dose seems to be appropriate for combinations with drugs that supposedly interfere with the effect of amitriptyline on inhibitory avoidance. A lower dose can be ineffective, as is the case of 5 mg/kg in the males of the present experiment, and a higher dose is unnecessary.

The number of subjects per group in the present experiment was high (20-24) in comparison with most similar experiments in the literature (10-12). This number is convenient for statistical purposes, but its generalized use is not advisable for non-scientific purposes.

The comparisons between training and test latencies of the same group showed that doses of 5 mg/kg or higher prevented inhibitory avoidance in all cases. Findings in our laboratory demonstrate that the impairing effect of amitriptyline on inhibitory avoidance is always observed with the pre-training administration and only sometimes with post-training administration (Ferrer-Añó, 2008; Urquiza, 2007). A greater influence of pre- vs post-training drug administration on memory has been reported for anticholinergic and anxiolitic drugs (Rush, 1988; Savic, Obradovic, Ugresic, & Bokonjic, 2005). It is well known that post-training administration avoids non-cognitive components of the effect of the drug (McGaugh, 1989; McGaugh & Roozendaal, 2009); nevertheless, a study of state dependent learning in which amitriptyline was administered before training to one of four groups, endorses the idea that the observed effects were due to memorization deficit (Arenas et al., 2006).

Acknowledgements

The «Ministerio de Ciencia y Tecnología» of Spain contributed to the funding support of the work reported here (Grant, PSI2008-06116). We also wish to thank Mr. Brian Normanly for his English editorial assistance.

References

Arenas, M.C., Vinader-Caerols, C., Monleón, S., Martos, A.J., Everss, E., Ferrer-Añó, A., & Parra, A. (2006). Are the effects of the antidepressants amitriptyline, maprotiline, and fluoxetine on inhibitory avoidance state-dependent? *Behavioural Brain Research, 166,* 150-158.

Azmi, N., Norman, C., Spicer, C.H., & Bennett, G.W. (2006). Effects of a neurotensin analogue (PD149163) and antagonist (SR142948A) on the scopolamine-induced deficits in a novel object discrimination task. *Behavioural Pharmacology 17*, 357-362.

Bammer, G. (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: A review and some new results. *Neuroscience & Biobehavioral Reviews 6*, 247-296.

Everss, E., Arenas, M.C., Vinader-Caerols, C., Monleón, S., & Parra, A. (2005). Piracetam counteracts the effects of amitriptyline on inhibitory avoidance in CD1 mice. *Behavioural Brain Research, 159,* 235-242.

Ferrer-Añó, A. (2008). *Estudio de la mediación del sistema histaminérgico en el efecto producido por la amitriptilina sobre la evitación inhibitoria en ratones*. Doctoral Thesis. Valencia: Universitat de València, CD-ROM.

Frazer, A. (1997). Pharmacology of antidepressants. *Journal of Clinical Psychopharmacology 17 Suppl 1*, 2S-18S.

Gold, P.E. (1986). The use of avoidance training in studies of modulation of memory storage. *Behavioral and Neural Biology 46*, 87-98.

McGaugh, J.L. (1989). Dissociating learning and performance: Drug and hormone enhancement of memory storage. *Brain Research Bulletin, 23*, 339-345.

McGaugh, J.L., & Roozendaal, B. (2009). Drug enhancement of memory consolidation: Historical perspective and neurobiological implications. *Psychopharmacology (Berl)*, *202*, 3-14.

Monleón, S., Vinader-Caerols, C., Arenas, M.C., & Parra, A. (2008). Antidepressant drugs and memory: Insights from animal studies. *European Neuropsychopharmacology*, *18*, 235-248.

Parra, A., Everss, E., Monleón, S., Vinader-Caerols, C., & Arenas, M.C. (2002). Effects of acute amitriptyline administration on memory, anxiety and activity in male and female mice. *Neuroscience Research Communications, 31,* 135-144.

Parra, A., Everss, E., Arenas, M.C., Vinader-Caerols, C., & Monleón, S. (2006). Amitriptyline administered after consolidation of inhibitory avoidance does not affect memory retrieval. *Psicothema, 18,* 514-518.

Rush, D.K. (1988). Scopolamine amnesia of passive avoidance: A deficit of information acquisition. *Behavioral and Neural Biology 50*, 255-274.

Savic, M.M., Obradovic, D.I., Ugresic, N.D., & Bokonjic, D.R. (2005). Memory effects of benzodiazepines: Memory stages and types versus binding-site subtypes. *Neural Plasticity, 12*, 289-298.

Snedecor, G.W., & Cochran, W.G. (1980). *Statistical methods*. The Iowa State University Press, Ames.

StatSoft, Inc. (2000). *Statistica for Windows* (Computer program manual). Tulsa, OK: StatSoft Inc.

Urquiza, A. (2007). *Estudio de la intervención del sistema colinérgico en el efecto producido por la amitriptilina en una tarea de evitación inhibitoria en ratones machos y hembras*. Doctoral Thesis. Valencia: Universitat de València, CD-ROM.