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Effects of anisomycin on inhibitory avoidance in male and female CD1 mice

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The antibiotic anisomycin inhibits protein synthesis, which much research has suggested is required for the formation of long-term memory. The present work studied the effects of acute subcutaneous administration of anisomycin on the consolidation of memory in an inhibitory avoidance task in CD1 mice of both sexes. The animals were separated by sex and randomly distributed into three groups: two groups were injected with 150 mg/kg anisomycin, one immediately after the training phase and the other 24 h later, while the control group received saline. The interval between training and test was four days. Anisomycin administrated immediately after training produced statistically significant impairment of memory, which was not observed when the drug was administered 24 h after training. No sex differences were observed in the effects of anisomycin. These results extend to female mice the memory impairing effects of anisomycin previously observed in males and endorse the hypothesis that the establishment of long-term memory depends on protein synthesis shortly after training.

Efectos de la anisomicina sobre la evitación inhibitoria en ratones CD1 machos y hembras. El antibiótico anisomicina inhibe la síntesis de proteínas, la cual muchos estudios indican que es necesaria para la formación de la memoria a largo plazo. En el presente trabajo se estudiaron los efectos de la administración aguda subcutánea de anisomicina sobre la consolidación de la memoria en una tarea de evitación inhibitoria en ratones CD1 de ambos sexos. Los animales fueron separados por sexo y distribuidos al azar en tres grupos: dos grupos fueron inyectados con 150 mg/kg de anisomicina, uno inmediatamente después de la fase de entrenamiento y el otro 24 h después, mientras que el grupo control recibió suero salino. El intervalo entre el entrenamiento y el test fue de cuatro días. La anisomicina administrada inmediatamente después del entrenamiento produjo un deterioro de memoria estadísticamente significativo, deterioro que no fue observado cuando el fármaco fue administrado 24 h después del entrenamiento. No se observaron diferencias de sexo en los efectos de la anisomicina. Estos resultados extienden a las hembras los efectos deteriorantes de la anisomicina sobre la memoria previamente observados en machos y respaldan la hipótesis de que el establecimiento de la memoria a largo plazo depende de la síntesis de proteínas poco después del entrenamiento.

Anisomycin, a well-established protein synthesis inhibitor (e.g., Cohen, Kaplan, Matar, Loewenthal, Kozlovsky, & Zoha, 2006), is an antibiotic that is widely used in experimental research about learning to disrupt memory, since it blocks both the late phase of long-term potentiation and that of long-term depression in the hippocampus (e.g., Kauderer & Kandel, 2000). It is also less toxic than other antibiotics and is effective for 2 h, after which protein synthesis resumes (Flood, Rosenzweig, Bennett, & Orme, 1973).

Inhibitory avoidance (also called passive avoidance) is commonly employed to study the pharmacology of memory in animals (e.g., Heise, 1981). This behavioural paradigm has also widely been applied in the study of memory formation (Izquierdo & Medina, 1997a). In the step-through version, the animal must avoid crossing to the dark compartment of a box and therefore evade receiving a shock to the foot (Bureš, Burešová, & Huston, 1983).

It is known that the administration of anisomycin weakens the retention of inhibitory avoidance learning in different experimental animals (Flood, Smith, Bennett, Alberti, Orme, & Jarvik, 1986; Freeman, Rose, & Scholey, 1995; Quevedo, Vianna, Roesler, de-Paris, Izquierdo, & Rose, 1999). This impairment is produced because protein synthesis is required for the establishment of an association between the context and the footshock (Frankland, Josselyn, Anagnostaras, Kogan, Takahashi, & Silva, 2004).

The majority of protocols in experimental research normally use male animals as experimental subjects. However, it is known that the responses of females can differ from those of males. This is evident not only in non-reproductive behaviours in intact

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animals, such as the inhibitory avoidance learning task (Everss, Arenas, Vinader-Caerols, Monleón, & Parra, 2005; Heinsbroek, Van Haaren, & Van de Poll, 1988; Monleón, Casino, Vinader-Caerols, & Arenas, 2001; Monleón, Urquiza, Arenas, Vinader-Caerols, & Parra, 2002), but also in subjects under the effects of drugs (Arenas et al., 2006; Everss et al., 2005; Monleón et al., 2002; Parra, Arenas, Monleón, Vinader-Caerols, & Simón 1999; Parra, Everss, Monleón, Vinader-Caerols, & Arenas, 2002; Parra, Everss, Arenas, Vinader-Caerols, & Monleón, 2006; Vinader-Caerols, Ferrer-Añó, Arenas, Monleón, & Parra, 2002). Although anisomycin has widely been used in studies on the consolidation of conditioned responses (e.g., Cohen et al., 2006; Flood et al., 1986; Freeman et al., 1995; Quevedo et al., 1999), as far as we are aware, no study has included females as experimental subjects. Thus, the aim of the present work was to study uncover differences between male and female CD1 mice with respect to the effects of acute subcutaneous administration of anisomycin on the consolidation of memory in the inhibitory avoidance task.

Methods

Animals

Male and female CD1 mice of 42 days of age, obtained from CRIFFA (Lyon, France), were used as experimental subjects. Animals were housed in groups of 5 in standard translucent plastic cages of 27 x 27 x 15 cm^3 (Panlab S.L., Barcelona, Spain), in a temperature-controlled room (21 ± 2 ºC), under a reversed light/dark cycle (lights off: 07:30-19:30, local time), with food and water available ad libitum. The task was always carried out during the dark phase of the light/dark cycle, after 11 days of acclimatization to the animal housing conditions. The experimental protocol and the use of animals were in compliance with the European Community's Council Directive of 24 November 1986 (86/609/EEC) and the Spanish Real Decreto 1201/2005.

Drugs

Anisomycin (Sigma-Aldrich Química, Madrid, Spain) was dissolved in a saline solution (0.9% NaCl) to obtain a dose of 150 mg/kg. Hydrochloric acid solution was employed to control the pH level (pH 7). All injections were administered subcutaneously 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 in the experiment. The cage is made of Perspex sheets and is divided into two compartments (15 × 9.5 × 16.5 cm^3 each one). The safe compartment is white and continuously illuminated by a light fixture fastened to the cage lid (24 V, 10 W, light intensity of 290 lux at floor level, measured with the Panlux Electronic2 photometer manufactured by GOSSEN, Nürnberg, Germany), whereas the «shock» compartment is dark, and made of black Perspex panels. The two compartments are separated by a partition with an automatically-operated sliding door at floor level. The floor is made of stainless steel bars with a diameter of 0.7 mm, and 8 mm apart.

Experimental procedures

Thirty-one male and 32 female CD1 mice were randomly distributed into three groups for each sex. This distribution guaranties a similar number of females of the different oestrous cycle stages in each experimental group. Animals were injected with 150 mg/kg anisomycin or saline immediately after the training phase and again at 24 h. The first group of each sex received physiological saline immediately after training and 24 h later (S-S); the second group was administered anisomycin immediately after training and physiological saline 24 h later (ANI-S); and the third group received physiological saline immediately after training and anisomycin 24 h later (S-ANI) (n= 9-11).

Mice were randomly subjected to the inhibitory avoidance task. Training and test phases began with a 90-s adaptation period to the apparatus in the light compartment. Following this, the door between the compartments was opened and the time taken to enter the dark compartment, defined as latency, was automatically measured in tenths of a second, and manually recorded after each trial. In the training phase, the door was opened and the mouse was allowed to stay in the light compartment for a maximum of 300 s. As soon as the animal entered the dark compartment the sliding door was closed and a foot-shock (0.5 mA for 5 s) was delivered through the grid floor. The inhibitory avoidance test was carried out four days later due to the protocol of drug treatment, following the same procedure as in the training phase, with the exception that no shock was delivered.

Data analysis

The inhibitory avoidance data were transformed into proportion (p= x/300) values and then to arcsin (arcsin$\sqrt{p}$) values, according to Snedecor & Cochran (1980). Analyses of variance for training and test were performed 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» software package, version 5.5 for windows (StatSoft, 2000).

Results

In the training phase, no significant differences were observed among the treatment groups [$F(2,55)= 0.62$, n.s.], which had been expected, since subjects were drug-free during this phase. Neither Sex factor [$F(1,55)= 1.14$, n.s.] nor the interaction of Sex X Treatment [$F(2,55)= 0.56$, n.s.] were statistically significant in this phase.

In the test phase, Treatment was statistically significant [$F(2,55)= 6.3$, $p<0.01$] and the Newman-Keuls test revealed that animals treated with anisomycin immediately after the training phase (ANI-S) produced shorter latencies than those treated only with saline (S-S) or those injected with anisomycin 24 h after the training phase (S-ANI) ($p<0.05$ and $p<0.01$, respectively). No sex differences were observed during the test [$F(1,55)= 1.15$, n.s.], while the interaction of Sex X Treatment [$F(2,55)= 0.41$, n.s.] was not statistically significant in this phase either.

The crossing latencies in the test phase were greater than those in the training session in all groups (S-S: $t_{21}= 8.75$; ANI-S: $t_{18}= 5.16$; S-ANI: $t_{19}= 12.72$; $p<0.01$ in all cases), which reflects learning of inhibitory avoidance (see figure 1).

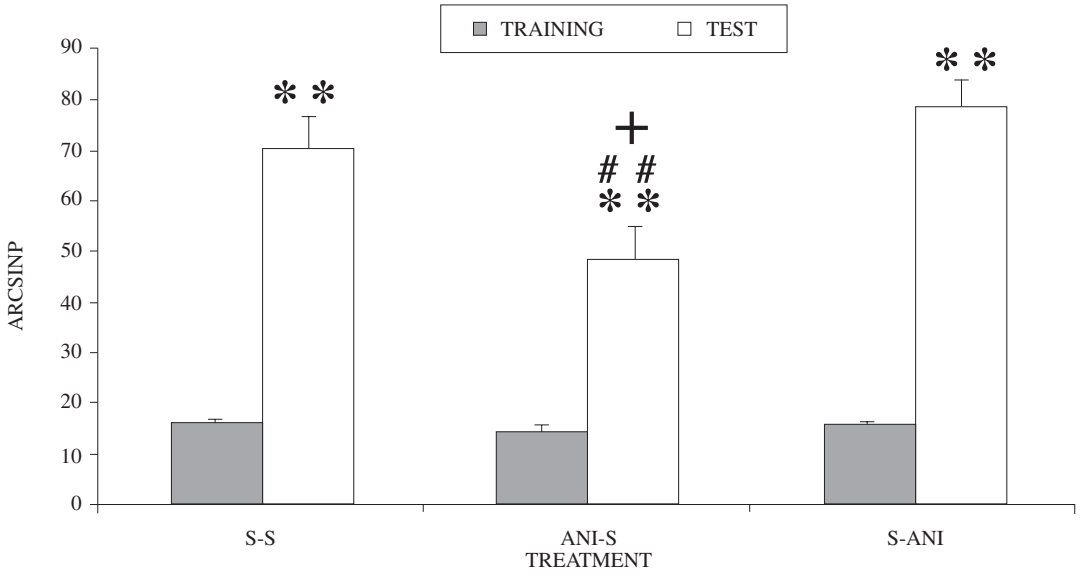

Figure 1. Effects of subcutaneous injections given immediately following training and 24 h later (S, saline; ANI, 150 mg/kg anisomycin) on step-through latencies of an inhibitory avoidance task in CD1 mice (n = 19-22, males and females together). Values are expressed as mean (+SEM) of square root of proportions (p = x/300) transformed to arcsin. **p<0.01 vs Training, +p<0.05 vs S-S group, ##p<0.01 vs S-ANI group

Discussion

In the present study, we investigated the effects of acute subcutaneous administration of anisomycin on the consolidation of memory in a step-through inhibitory avoidance task in male and female CD1 mice. The administration of anisomycin immediately after the training phase produced statistically significant memory deterioration, as illustrated by the figure. However, this impairing effect of anisomycin was not observed when the drug was administered 24 h after training, supposedly because the process of long-term memory formation in this learning task has already been completed (Izquierdo & Medina, 1997b). This lack of effect on retention is similar to that found, in the same behavioural context, with the antidepressants fluoxetine (Monleón et al., 2002) and amitriptyline (Parra et al., 2006).

In spite of the widespread use of anisomycin in experimental research exploring learning, we have failed to locate any study in which sex has been analyzed as an independent variable. In the field of psychopharmacology, studies tend to use only male animals as experimental subjects, since there is a general belief that data obtained in males have a lower variability than those obtained in females. Nevertheless, in a number of experiments carried out in our laboratory, we observed that there were no sex differences in the variability of responses obtained during an active avoidance task (Parra et al., 1999). Thus, in the present work, the study of the effects of anisomycin on inhibitory avoidance in mice was extended to females. Although no differences were observed between the sexes in these effects, the results should be interpreted as proof of a generalization among previously published data obtained exclusively in males, rather than as a negative result. Nevertheless, sex differences in the effects of anisomycin on inhibitory avoidance could be observed in future experiments if some variables were taken into account: the doses of the drug, the stage of the oestrous cycle, or the timing of drug administration.

Our results endorses the use of anisomycin as a reliable animal model of amnesia in male and female mice, especially when administered in combination with other drugs that impair or enhance memory and whose effects could be sex dependent.

In summary, the present results demonstrate that the memory-impairing effects of anisomycin previously observed in males are also noted in females, and bolster the hypothesis that the establishment of long-term memory depends on the protein synthesis that takes place shortly after training.

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