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EFFECTS OF CHLOROGENIC ACID ON LEARNING AND MEMORY IN RATS

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ABSTRACT

Chlorogenic acid (CA) is present in foods such as coffee, kiwis, apples, aronia. etc. PURPOSE: This study investigated the effect of CA on learning and memory in male Wistar rats. METHODS: CA was administrated orally (20 mg/kg as a 10 ml/kg solution) to different groups of rats for 7, 14, 21 and 30 days. Control rats were respectively treated with saline (10 ml/kg). At the end of each experimental period, learning and memory were evaluated using the one-way passive avoidance task (step through) and the two-way active avoidance task (shuttle box). RESULTS: Administered for 7 and 14 days, CA had no significant effects on rat behavior in both tasks. After 21 and 30 days of administration, in the step through task CA significantly prolonged the latency time during the retention tests on the 3rd and 24th hour and increased the percentage of rats reaching the learning criterion (remaining in the illuminated compartment for at least 180 sec). After 21 and 30 days of administration, in the shuttle box task CA significantly increased the number of avoidances on the 1st and 2nd training days as well as at the retention test (24 h after the 2nd training session). CONCLUSIONS: CA improved learning and memory processes in young/healthy rats.

Key words: chlorogenic acid, learning, memory, rats

INTRODUCTION

Chlorogenic acid (CA) is a phenolic acid present in foods such as coffee, beans, potatoes, apples, kiwis, plums and aronia fruits. In the European diet, coffee is a predominant source of CA. Since the discovery that polyphenolics are able to cross the blood-brain barrier there has been an intense interest in the neuroprotective effects of polyphenols, which are powerful antioxidants in vitro (1). CA has been shown to improve memory in a model of scopolamine-induced amnesia in mice (2). To date, there has been only a limited number of studies investigating the effects of polyphenol-rich foods on cognition in young/healthy animals (3).

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The aim of the present study was to investigate the effect of CA on learning and memory in male young/healthy Wistar rats using the one-way passive avoidance task (step through) and the two-way active avoidance task (shuttle box).

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (200-240 g at the beginning of experiments) were housed in polypropylene boxes with free access to food and water. The experiments were carried out according to the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences, in compliance with the national policies and the EEC Directive of 1986 (86/609/EEC).

The step through task was performed on 96 rats divided into 8 groups of 12 animals each. The animals were treated orally through an orogastric cannula in the course of 7 days (one week), 14 days (two weeks), 21 days (three weeks) or 30

days (one month). There were two groups of rats for each treatment period: Control and CA. Rats from CA groups were treated with CA (Sigma-Aldrich, Germany) at a dose 20 mg/kg as a solution (volume 10 ml/kg). The control groups were treated with saline (10 ml/kg).

The shuttle box task was performed on other 96 rats divided into 8 groups of 12 animals. There were Control and CA groups of rats for each treatment period (7, 14, 21 and 30 days). The treatment of animals was the same as described for the step through task.

One-way passive avoidance task – step through

In the passive avoidance task in order to avoid a mild foot shock, the rat must learn to remain in a brightly lit compartment and not enter the preferred dark compartment. The passive avoidance task was carried out using a step through apparatus. The apparatus had two chambers: a dark one (30 x 30 x 30 cm) with metal grid floor and a brightly illuminated (100 W) one (8 x 7 x 30 cm). The two chambers were separated by a guillotine-type door. One training trial and two retention tests were conducted according to the method of Gozzani and Izquierdo (4). The training trial started by placing the rat in the brightly lit compartment. Once the rat had entered the dark compartment, the guillotine door was closed and an electric shock (0.30-0.35 mA for 3 sec) was delivered to the animal through the grid floor. Each rat underwent one trial. Retention tests (no shocks) were performed 3 h and 24 h after the acquisition trial. At that time, the animal was returned to the illuminated compartment, and the step-through latency was estimated by measuring the time (latency time) for the rat to move to the dark compartment. A latency of at least 180 sec was used as a criterion for learning. The last CA application was 60 min before the training trial. CA was not given to the rats before the two retention tests. Before each test, the apparatus was wiped clean and dried.

Two-way active avoidance task – shuttle box

In the shuttle box task, in order to avoid a mild foot shock, the rat must learn to shuttle from one end of the box to the other every time a warning signal (conditioned stimulus) is presented. The two-way active avoidance task was carried out after the method of Buresova and Bures modified by Petkov et al. (5). The shuttle box apparatus (50 x 29 x 21 cm) was divided in two equal compartments provided with a round opening in the centre. Light (20 W switched on alternately in the two compartments) was used as a conditioned stimulus. The conditioned stimulus was switched on in the part of the cage opposite to the part in which the rat was located at the end of the inter-trial period. The unconditioned stimulus was an electric shock (0.5 mA, 50 Hz) applied to the grid floor for 12 sec. The conditioned stimulus preceded the onset of the unconditioned stimulus by 9 sec and continued during the action of the unconditioned stimulus. An avoidance response (correct response) was recorded when the animal avoided the unconditioned stimulus within 9 sec after the onset of the conditioned stimulus. The inter-trial interval was 9 sec. There were two learning sessions (on two consecutive days) each consisting of 50 trials for each rat. A retention test was carried out 24 h after the second training session: the light stimulus was applied for 9 sec and was followed by 2 sec electric shock. The inter-trial interval was 9 sec. CA was applied 60 min before the two learning sessions and was not given to the animals before the retention test. The number of avoidances was recorded in the shuttle-box learning tests (on the 1^{st} and 2^{nd} training day) and retention test (24 hours after the 2^{nd} training day). Before each test, the apparatus was wiped clean and dried.

Statistical analysis

Results are presented as mean ± S.E.M. Data were analyzed using the Students's *t*-test. A level of $p<0.05$ was considered significant. Analysis of the data for the learning criterion was performed using χ^2 test. GraphPad Prism statistical software was used.

RESULTS

One-way passive avoidance task – step through

Applied to rats for 7 and 14 days, CA did not affect the latency time and the learning criterion at the retention tests on the 3^{rd} and 24^{th} hour **(Figure 1A-B, Table 1**).

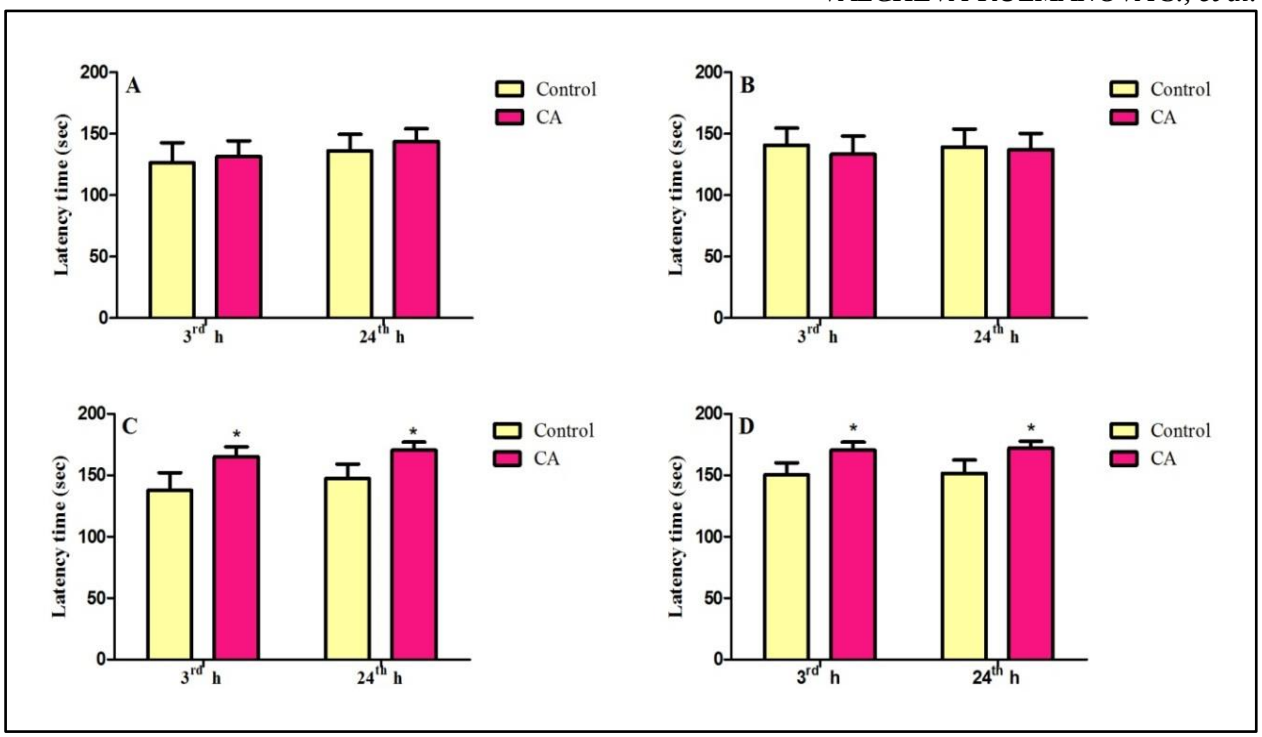

Figure 1. Latency time during the retention tests on the 3rd and 24th h in the step through task after treatment of rats with chlorogenic acid (CA) for 7 days (panel A), 14 days (panel B), 21 days (panel C) and 30 days (panel D). Results are presented as mean ± S.E.M.; n = 12; *p<0.05 vs. Control

Table 1. *Effect of chlorogenic acid (CA) applied for 7, 14, 21 and 30 days on the learning criterion of rats during the retention tests on the 3rd and 24th h after training in the step through task*

Groups	Learning criterion (% of rats)							
	7 days		14 days		21 days		30 days	
	3rd h	24th h	3rd h	24th h	3rd h	24th h	3rd h	24th h
Control	50	50	50	50	50	57	50	58
CA	42	42	42	42	67	67	83	83

After 21 days of administration, CA significantly prolonged the latency time at the retention tests on the 3rd (p<0.05) and 24th hour (p<0.05) **(Figure 1C)** but had no significant effect on the percentage of rats that had reached the learning criterion **(Table 1).** Applied for 30 days, CA significantly prolonged the latency time at the retention tests on the 3rd (p<0.05) and 24th hour (p<0.05) **(Figure 1D)** without significantly affecting the percentage of rats that had reached the learning criterion **(Table 1).**

Two-way active avoidance task – shuttle box

After the treatment periods of 7 and 14 days, CA had no significant effect on the number of avoidances on the 1st and the 2nd training days and at the retention test **(Figure 2A-B)**. After 21 days of administration, CA significantly increased the number of avoidances on the 1st training day (p<0.05), on the 2nd training day (p<0.01) and at the retention test (p<0.05) in comparison with the saline-treated controls **(Figure 2C).** After the 30 days treatment period, CA significantly increased the number of avoidances on the 1st training day (p<0.05), on the 2nd training day (p<0.01) and at the retention test (p<0.01) as compared to the saline-treated controls **(Figure 2D).**

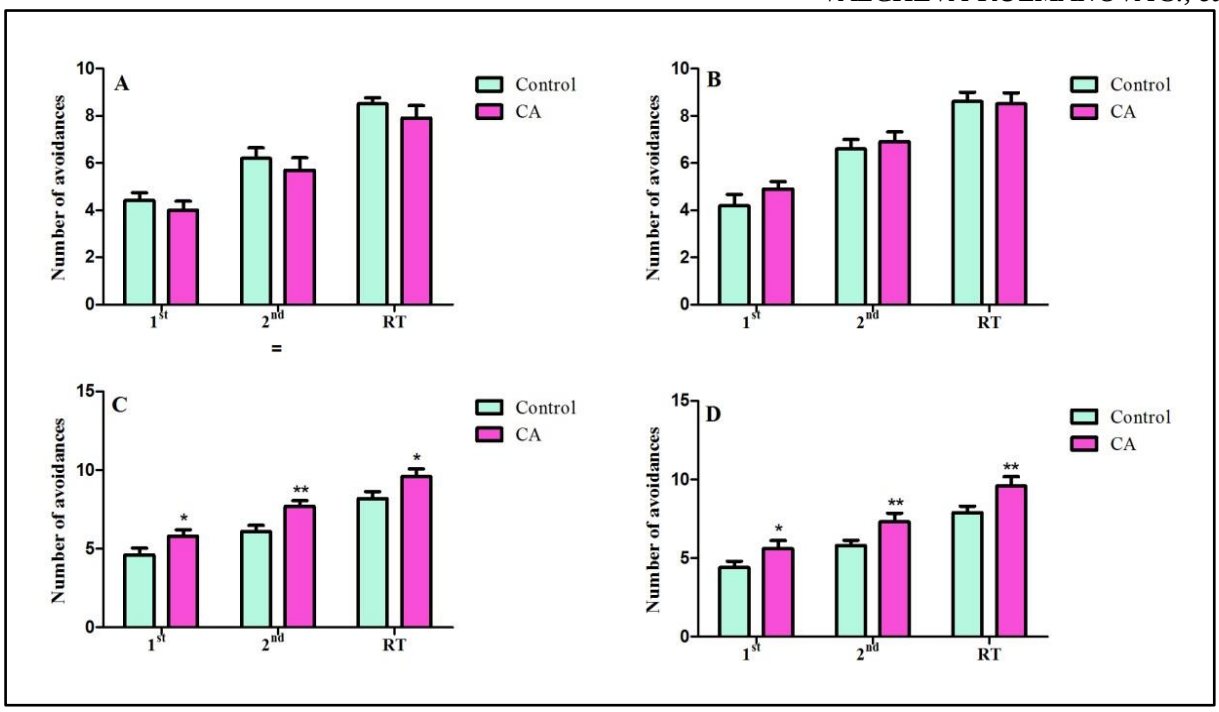

Figure 2. Number of avoidances of rats during the 1st and 2nd training sessions and during the retention test in the shuttle box task after treatment with chlorogenic acid (CA) for 7 days (panel A), 14 days (panel B), 21 days (panel C) and 30 days (panel D). Results are presented as mean ± S.E.M.; n = 12; *p<0.05 vs. Control; **p<0.01 vs. Control

DISCUSSION

In this study, the learning and memory processes of rats tested by the step through and the shuttle box tasks were markedly improved by CA administered to the animals for 21 and 30 days. There are literature data that polyphenols accumulate in the brain following long-term consumption (6). Studies have demonstrated antioxidant actions of polyphenols in the brain. This classical antioxidant activity probably does not account for all biological effects of polyphenols in vivo, particularly in the brain, where they are found at only very low concentrations (7). From the review of literature, it is clear that plant polyphenolic substances might improve memory by several mechanisms: antioxidant activity, vascular effects, activation of signaling pathways and inhibition of acetylcholinesterase activity (7). The exact mechanism of CA-induced improvement of memory and learning performance in rats remains to be elucidated.

CONCLUSION

The results from the step through and shuttle box tasks showed that CA, administered to young/healthy male rats for periods of 21 and 30 days, improved learning and memory.

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