Neuroscience 202 (2012) 384–395

CHRONIC CAFFEINE CONSUMPTION PREVENTS COGNITIVE DECLINE FROM YOUNG TO MIDDLE AGE IN RATS, AND IS ASSOCIATED WITH INCREASED LENGTH, BRANCHING, AND SPINE DENSITY OF BASAL DENDRITES IN CA1 HIPPOCAMPAL NEURONS

S. VILA-LUNA,[a] S. CABRERA-ISIDORO,[b] L. VILA-LUNA,[a] I. JUÁREZ-DÍAZ,[b,c] J. L. BATA-GARCÍA,[a] F. J. ALVAREZ-CERVERA,[a] R. E. ZAPATA-VÁZQUEZ,[d,e] G. ARANKOWSKY-SANDOVAL,[a] F. HEREDIA-LÓPEZ,[a] G. FLORES[b] AND J. L. GÓNGORA-ALFARO[a,d]*

[a]*Departamento de Neurociencias, Centro de Investigaciones Regionales (CIR) "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán (UADY), Avenida Itzáes 490 × 59, Mérida, Yucatán 97000, Mexico*

[b]*Laboratorio de Neuropsiquiatría, Instituto de Fisiología, Universidad Autónoma de Puebla, 14 Sur 6301, Puebla 72570, México*

[c]*Laboratorio de Fisiología, Facultad de Estomatología, Universidad Autónoma de Puebla, 31 Poniente 1304, Puebla 72410, México*

[d]*Facultad de Medicina, Universidad Autónoma de Yucatán, Avenida Itzáes 498 × 59-A, Mérida, Yucatán 97000, Mexico*

[e]*Unidad Médica de Alta Especialidad, Instituto Mexicano del Seguro Social, Ex-terrenos El Fénix, Mérida, Yucatán 97150, Mexico*

Abstract—Chronic caffeine consumption has been inversely associated with the risk of developing dementia and Alzheimer's disease. Here we assessed whether chronic caffeine treatment prevents the behavioral and cognitive decline that male Wistar rats experience from young (≈3 months) to middle age (≈10 months). When animals were young they were evaluated at weekly intervals in three tests: motor activity habituation in the open field (30-min sessions at the same time on consecutive days), continuous spontaneous alternation in the Y-maze (8 min), and elevated plus-maze (5 min). Afterward, rats from the same litter were randomly assigned either to a caffeine-treated group (n=13) or a control group (n=11), which received only tap water. Caffeine treatment (5 mg/kg/day) began when animals were ≈4 months old, and lasted for 6 months. Behavioral tests were repeated from day 14 to day 28 after caffeine withdrawal, a time period that is far in excess for the full excretion of a caffeine dose in this species. Thirty days after caffeine discontinuation brains were processed for Golgi-Cox staining. Compared with controls, we found that middle-aged rats that had chronically consumed low doses of caffeine (1) maintained their locomotor habituation during the second consecutive day exposure to the open field (an index of non-associative learning), (2) maintained their exploratory drive to complete the conventional minimum of nine arm visits required to calculate the alternation performance in the Y-maze in a greater proportion, (3) maintained their alternation percentage above chance level (an index of working memory), and (4) did not increase the anxiety indexes assessed by measuring the time spent in the open arms of the elevated plus maze. In addition, morphometric analysis of hippocampal neurons revealed that dendritic branching (90–140 μm from the soma), length of 4th and 5th order branches, total dendritic length, and spine density in distal dendritic branches were greater in the basal but not the apical dendrites of CA1 pyramidal neurons from rats chronically treated with caffeine, in comparison with their age- and littermate-matched controls. Altogether, the present findings strengthen the epidemiological observations suggesting that prolonged caffeine intake prevents the cognitive decline associated with aging, and open the possibility that this process could be mediated by promoting the growth of dendrites and spines in neurons of the adult mammalian brain.

Key words: caffeine, methylxanthines, aging, memory, neuroprotection, dendritic growth.

During the past decade evidence that moderate consumption of coffee and other caffeinated beverages delays the onset of dementia (Eskelinen and Kivipelto, 2010) and reduces the risk of developing Alzheimer's disease (AD) in elderly people (Maia and de Mendonça, 2002; Lindsay et al., 2002; Eskelinen and Kivipelto, 2010) has accumulated. In addition, epidemiological surveys performed in elderly populations have found that habitual caffeine consumption shows a significant association with a lower cognitive decline in men (van Gelder et al., 2007) and women (Santos et al., 2010b), a better capacity for word recall and verbal retrieval in women (Johnson-Kozlow et al., 2002; Ritchie et al., 2007), and a better long-term memory for word recall, and faster motor speed in both sexes (Hameleers et al., 2000). However, not all published reports agree with the above findings, an incongruity that has been attributed to the lack of a standard methodology among studies (Rosso et al., 2008; Santos et al., 2010a). These discrepancies have given impetus to animal studies designed to assess the neuroprotective actions of chronic caffeine administration under controlled experimental conditions.

Thus, the possibility that long-term caffeine consumption could prevent AD has been supported by experiments with transgenic mice (APPsw) bearing a mutated form of human amyloid-β (Aβ) linked to familial AD, in which long-term treatment with caffeine at a dose equivalent to that contained in five cups of coffee per day, afforded protection against cognitive impairment during aging and prevented Aβ deposition in the hippocampus (Arendash et al.,

*Corresponding author. Tel: +52 999-924-6412; fax: +52 999-923-6120.
E-mail address: jlgongoralf@gmail.com (J. L. Góngora-Alfaro).

Abbreviations: AD, Alzheimer's disease; A_1R, adenosine A1 receptor; $A_{2A}R$, adenosine A_{2A} receptor; Aβ, amyloid-β; DG, dentate gyrus; EPM, elevated plus maze; LTP, long-term potentiation; OF, open field; RM-ANOVA, repeated measures analysis of variance; SAB, spontaneous alternation behavior.

0306-4522/12 $36.00 © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2011.11.053

2006). Moreover, when aged APPsw mice already demonstrating cognitive impairment were treated with a chronic caffeine regime, they showed a significant improvement in working memory that was associated with a significant reduction of Aβ deposits in the hippocampus and entorhinal cortex (Arendash et al., 2009). In addition, other studies have demonstrated that wild-type mice pretreated with caffeine (30 mg/kg/day) during four days become resistant to the memory deficits caused by the i.c.v. injection of Aβ peptide fragment 25–35 (Dall'Igna et al., 2007).

However, studies aimed at evaluating whether chronic caffeine treatment prevents the cognitive deterioration that occurs during aging in healthy animals have given inconsistent results. Thus, in one report 18-month old male mice were evaluated after 12 months of caffeine ingestion through their drinking water (≈125 mg/kg/day), and it was found that their working memory in an object recognition task was better than that of their age-matched controls, and similar to the performance of 6-month old animals (Costa et al., 2008). In contrast, another study that evaluated 16-month old male mice following 10 months of caffeine treatment (≈60 mg/kg/day) failed to find improved performance in a battery of cognitive and motor tests, in comparison with animals that consumed only water (Arendash et al., 2009). These last findings agree with those of an earlier study performed in 10-month old male rats that consumed a caffeine solution (0.1 mg/ml) during 12 months, and showed no difference in the maintenance of their acquired spatial memory in the Lashley III maze when compared with control animals (Espinola et al., 1997). It should be noted that in all these studies behavioral testing was performed while the animals were still under caffeine treatment. We are not aware of any study aimed at assessing whether the putative cognitive benefits afforded by chronic caffeine treatment extend far beyond its consumption period. Such experimental design would be necessary to disclose whether any memory improvement seen after prolonged periods of caffeine ingestion are caused by a true neuroprotective action, and not by its acute cognitive normalizing effects, which have been consistently observed in animal models of memory dysfunction (Takahashi et al., 2008).

Another point that deserves consideration is the fact that high doses have been used in the studies that have tested the impact of chronic caffeine intake in healthy animals (Costa et al., 2008; Arendash et al., 2009). This has likely produced brain concentrations affecting multiple molecular targets other than adenosine receptors (Yu et al., 2009), whose blockade by caffeine has been linked to its protective effects against memory dysfunction (Takahashi et al., 2008; Cunha and Agostinho, 2010). However, some reports have supported the possibility that lower doses of caffeine can produce beneficial effects on brain functioning. Thus, APPsw transgenic mice that received a single dose of caffeine, as low as 5 mg/kg, had a significant decrease of Aβ outflow measured by microdialysis in the interstitial space of the hippocampus (Cao et al., 2009). Another study found that healthy male rats that consumed a daily caffeine dose of 5 mg/kg during 6 months, followed by a withdrawal period of at least 2 weeks, developed a greater resistance to the catalepsy induced with the dopaminergic antagonist haloperidol, suggesting that this chronic caffeine schedule produced perdurable changes in brain functioning, not attributable to the presence of caffeine or its metabolites in the cerebral tissue (Góngora-Alfaro et al., 2009).

Consequently, the present study was designed to assess whether chronic intake of a low caffeine dose by healthy adult rats, followed by a 2-week withdrawal period (a) prevents the progressive motor impairment that occurs during normal aging (Willig et al., 1987; Altun et al., 2007); (b) preserves the habituation of motor activity during repeated exposure to the open field (OF), which is a form of non-associative learning (Leussis and Bolivar, 2006) that deteriorates during the aging process (Fraley and Springer, 1981); (c) prevents the aging-associated working memory decline in the Y-maze (Willig et al., 1987; Stone et al., 1992); and (d) favors the development of anxiety-like states as those produced by either acute (Pellow et al., 1985) or chronic (El Yacoubi et al., 2000) high doses of caffeine. In addition, the morphometric analysis of Golgi-Cox stained hippocampal neurons from control and caffeine-treated rats was made because caffeine induces spontaneous firing oscillations in the hippocampus (Pietersen et al., 2009), a nucleus involved in the control of a variety of motor and exploratory behaviors, and previous studies have shown that chronic caffeine treatment causes morphological changes in vertebrate pyramidal neurons (Burgess and Monachello, 1983; Juárez-Méndez et al., 2006).

EXPERIMENTAL PROCEDURES

Animals

The present study was performed on 24 male Wistar rats from five litters bred in our facilities. Only males were used because there is evidence that estrogens may counteract some neuroprotective actions of caffeine in rodents (Xu et al., 2006). They were acclimatized to the laboratory environment for at least 1 week before any experimental manipulation took place, with 12:12 h light/dark cycles (lights on at 07:00), room temperature of 23±2 °C, and food and water *ad libitum*. Groups of two to four animals were housed in acrylic cages (length, 42 cm; width, 32 cm; height, 17.5 cm) with the same partners throughout the experimental period. All behavioral tests were carried out during light hours (09:00–15:00 h), in a room with controlled temperature (23±2 °C), and a fixed light intensity of approximately 100 lx. When testing began rats had an average weight of 312±8 g, and at the moment of the last experiment their weights averaged 489±14 g. This study was approved by the Institutional Bioethics Committee of the CIR-UADY, and all efforts were made to minimize animal discomfort according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the USA, 1996 revised version.

Treatments

A similar number of animals from the same litter was assigned (see Table 1) to either a caffeine-treated group (5 mg/kg/day, n=13) or a control group (n=11) that received only tap water. The caffeine solution was freshly prepared every day and administered *ad libitum* through the drinking water. In order to ensure that the animals ingested the expected 5 mg/kg daily dose of caffeine its