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Caffeine impairs long-term memory in the step-through passive avoidance task in mice

Overwhelming evidence indicates that caffeine (CAF; 1,3,7-trimethylxanthine) is a psycho-active substance ameliorating brain functioning in both experimental and clinical studies (for review see 3, 10). CAF increases energy metabolism throughout the brain, activates noradrenaline neurons and seems to affect the local release of dopamine. Therefore, this methylxanthine is considered as a mild agent ameliorating brain functioning and improving memory in humans; however, its psychostimulant action is often subtle and difficult to present (10). CAF is a common ingredient in analgesic drugs, appetite-suppressant drugs, and stimulant adjuvant drugs increasing physical and mental performance (3, 10). Moreover, CAF is a widely consumed methylxanthine derivative as coffee, energizing drinks and candies. Although the precise molecular mechanism of action of CAF remains unclear, this psycho-active compound at micromolar physiological concentrations possesses adenosine receptor antagonizing properties, being a non-specific adenosine A_1 and A_{2A} receptor antagonist. Moreover, CAF (at millimolar concentrations) due to its phosphodiesterase inhibiting and Ca^{2+} releasing properties provides an increase in cAMP content and A-kinase activity in neurons, which may also be responsible for its psychostimulant activity *in vivo* (1, 3, 10).

The aim of this study was to determine the effect of acute exposure to CAF on long-term memory in the step-through passive avoidance task. This test allowed the evaluation of effect of CAF on acquisition and consolidation of memory patterns in animals (8, 9, 11).

MATERIAL AND METHODS

A n i m a l s. All experiments were performed on male Albino Swiss mice, kept in colony cages with free access to food and tap water, under standardized housing conditions. The animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse participated only in one experiment. All tests were performed between 9.00 a.m. and 2.00 p.m. to minimize confounding effects of circadian rhythms. The experimental protocols and procedures described in this manuscript were approved by the Local Ethics Committee at the Medical University of Lublin (License no.: 534/2005/569/2005).

D r u g. Caffeine-sodium benzoate (CAF; Sigma, St. Louis, MO, USA) was dissolved in saline and administered intraperitoneally (i.p.) in a volume of 0.005 ml/g body weight, at 30 min. before the test. Doses of CAF tested in this study refer to the pure methylxanthine and were based on previous

experiments evaluating the effects of acute and chronic (for 14 days) treatments of CAF on the threshold for electroconvulsions in mice (2, 4).

Step-through passive avoidance task. Animals were administered with increasing doses of CAF (11.55 to 92.4 mg/kg) on the first day before training. Subsequently, the animals were placed in an illuminated box (10 x 13 x 15 cm) connected to a larger dark box (25 x 20 x 15) equipped with an electric grid floor. Entrance of the animals to the dark box was punished by an adequate electric footshock (0.6 mA for 2 s). On the next day (24 h later), the pre-trained animals were put again into the illuminated box and observed for up to 180 s. The time since the moment the mice entered the dark box, was noted and subsequently, the medians of retention time with 25th and 75th percentiles were calculated. Mice that avoided the dark compartment for 180 s were considered to remember the task (11).

Statistics. Qualitative variables from the step-through passive avoidance task were statistically evaluated using Kruskal-Wallis nonparametric ANOVA test followed by the *post-hoc* Dunn's multiple comparisons test. Additionally, linear regression analysis according to Glantz and Slinker (5) was used to assess the trend between the increasing doses of CAF and their resultant reduction in the retention time in animals.

RESULTS

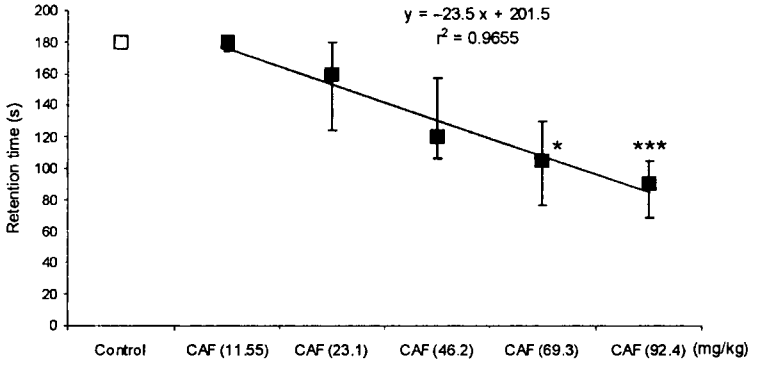
The systemic (i.p.) administration of CAF caused significant memory deficits in animals challenged with the step-through passive avoidance task (Table 1). The level of decrease of retention times in animals in the passive avoidance task was associated with an increase in CAF dosage (Table 1). It was found that CAF (92.4 mg/kg) considerably shortened the retention time of mice from 180 s to 90 s ($p<0.001$; Table 1; Fig. 1). Likewise, CAF at a dose of 69.3 mg/kg shortened significantly the retention time in animals from 180 s to 105 s ($p<0.05$; Table 1; Fig. 1). CAF at low doses of 23.1 and 46.2 mg/kg also shortened the retention time in mice, although the results did not attain statistical significance. Only, CAF at a dose of 11.55 mg/kg did not disturb long-term memory in animals subjected to the step-through passive avoidance task (Table 1).

Table 1. Effect of caffeine on long-term memory in the step-through passive avoidance task in mice

Treatment (mg/kg)	Retention time (s)
Control	180 (180; 180)
CAF (11.55)	180 (173.8; 180)
CAF (23.1)	160 (123.8; 180)
CAF (46.2)	120 (106.3; 157.5)
CAF (69.3)	105 (76.3; 130)*
CAF (92.4)	90 (68.8; 105)***

Data are presented as medians of retention time (with 25th and 75th percentiles in parentheses) of at least 8 determinations. Caffeine (CAF) was administered i.p., 30 min prior to the test. Statistical evaluation of the data was performed with Kruskal-Wallis nonparametric ANOVA test followed by the *post-hoc* Dunn's test. *$p<0.05$, and ***$p<0.001$ vs. control (vehicle-treated) animals

Linear regression analysis of increasing doses of CAF and their resultant reduction in the retention time in animals revealed the existence of a close relationship between the analyzed data. The equation of dose-response relationship between CAF doses and their decrease in the retention time in the step-through passive avoidance task was $y = -23.5\ x + 201.5$ ($r^2 = 0.9655$); where y is the retention time (in s), x – the CAF dose, and r^2 – the coefficient of determination (Fig. 1). Since $r^2 = 0.9655$, one can conclude that 96.55% of the total variance of the dependent variable y (retention time reduction) is explained by the linear regression model and its equation ($y = -23.5\ x + 201.5$). In other words, the points creating the line were good-to-fit.



Results are presented as median retention times (in s; with 25th and 75th percentiles as the error bars). Data were statistically analyzed with Kruskal-Wallis nonparametric ANOVA test followed by the *post-hoc* Dunn's test. *p<0.05, and ***p<0.001 vs. control (vehicle-treated) animals. Additionally, the doses of CAF were plotted graphically (X-axis) against the retention time (Y-axis) in the step-through passive avoidance task in mice. Least-squares linear regression allowed the determination of the equation of dose-response relationship for CAF evaluated in the passive avoidance task in mice, which was $y = -23.5\ x + 201.5$ ($r^2 = 0.9655$). The line on the graph represents a trend existing between CAF doses and their resultant decrease in retention time

Fig. 1. Effect of caffeine (CAF) on long-term memory in the step-through passive avoidance task in mice

DISCUSSION

The results presented herein indicate clearly that CAF significantly disturbed long-term memory in the animals. The dose-response relationship analysis with linear regression revealed that CAF dose-dependently shortened the retention time in the animals subjected to the step-through passive avoidance task. The observed changes in long-term memory in mice after CAF administration could be explained based on a stimulant activity of the drug. It is possible that psychostimulant activation of the brain by CAF may change physiological processes associated with acquisition and consolidation of memory patterns. So, the animals, after being exposed to aversive stimulation in the step-through passive avoidance task, did not remember this task on the following day, because the testing was not remembered by the animals. In such a situation, CAF may produce dissociation of memory patterns in the brain. This hypothesis could, at least in part, explain the observed changes in long--term memory in animals. Moreover, our findings indicating that CAF significantly impaired long--term memory in mice, are generally in agreement with those reported recently, documenting that

theophylline (1,3-dimethylxanthine; a compound structurally and chemically similar to CAF) has also disturbed memory and learning processes in developing mice (7). On the other hand, it has recently been observed that some antiepileptic drugs possessing antinociceptive properties produced distinct impairment of long-term memory in mice challenged with the step-through passive avoidance task (8). Noticeably, although CAF is added to analgesic drugs in order to enhance their action, the methylxanthine *per se* does not exert the antinociceptive influence. Another fact deserves more attention and should be discussed here. Since CAF increases dose-dependently the locomotor activity of animals (6), the mice could enter the dark box chaotically (by accident), and thus, producing significant impairment of long-term memory. This hypothesis is not excluded and also should be borne in mind when one can try to explain the observed impairment of long-term memory in mice. Noteworthy, the effects of CAF on learning, memory, performance and coordination in humans are rather related to the methylxanthine action on arousal, vigilance and fatigue (3). If extrapolating the results from this preclinical study to clinical conditions, a caution is advised for individuals taking CAF in high doses, because of the fact that CAF may not ameliorate acquisition and retrieval in memory processes, but paradoxically, the methylxanthine may decrease cognitive functioning and disturb memory. Since CAF is available and commonly consumed by children and adolescents in the form of energizing drinks and candies, the consumption of CAF may negatively affect their cognitive functioning. However, this fact needs clinical verification in further studies.

CONCLUSIONS

1. In this study, a close relationship between increasing doses of CAF and their resultant changes in long-term memory was observed.

2. The utmost caution is advised for children and adolescent consuming ingredients containing CAF (e.g. caffeinated beverages, candies) because CAF may disturb physiological processes related to learning and memory in the brain.

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REFERENCES

1. Choi O. H. et al.: Caffeine and theophylline analogues: correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sci., 43, 387, 1988.
2. Czuczwar S. J. et al.: Influence of different methylxanthines on the anticonvulsant action of common antiepileptic drugs in mice. Epilepsia, 31, 318, 1990.
3. Fredholm B. B. et al.: Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev., 51, 83, 1999.
4. Gasior M. et al.: Anticonvulsant activity of phenobarbital and valproate against maximal electroshock in mice during chronic treatment with caffeine and caffeine discontinuation. Epilepsia, 37, 262, 1996.
5. Glantz S. A., Slinker B. K.: Primer of Applied Regression & Analysis of Variance. Second edition. McGraw-Hill Inc., New York 2001.
6. Hilakivi L. A. et al.: Effects of caffeine on social behavior, exploration and locomotor activity: interactions with ethanol. Life Sci., 44, 543, 1989.
7. Hirose M. et al.: Theophylline impairs memory/learning in developing mice. Brain Dev., 26, 448, 2004.

8. Łuszczki J.J. et al.: Tiagabine synergistically interacts with gabapentin in the electroconvulsive threshold test in mice. Neuropsychopharmacology, 28, 1817, 2003.
9. Łuszczki J. J. et al.: Pharmacological and behavioral characteristics of interactions between vigabatrin and conventional antiepileptic drugs in pentylenetetrazole-induced seizures in mice: an isobolographic analysis. Neuropsychopharmacology, 30, 958, 2005.
10. Nehlig A. et al.: Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res. Rev., 17, 139, 1992.
11. Venault P. et al.: Benzodiazepines impair and beta-carbolines enhance performance in learning and memory tasks. Nature, 321, 864, 1986.

SUMMARY

The aim of this study was to determine the effect of single administration of caffeine (CAF) on long-term memory in mice challenged with the step-through passive avoidance task. Results indicate that CAF (administered intraperitoneally, 30 min. before the test) at doses of 69.3 and 92.4 mg/kg significantly impaired long-term memory by shortening the retention time in animals from 180 s (control) to 105 s ($p<0.05$), and 90 s ($p<0.001$), respectively. In contrast, CAF at 11.55, 23.1 and 46.2 mg/kg did not alter significantly long-term memory in animals. Linear regression analysis revealed a trend between the increasing doses of CAF and their resultant decrease in retention times, indicating that CAF impaired dose-dependently long-term memory in the step-through passive avoidance task. Based on this preclinical study, one can conclude that CAF at high doses may also impair learning and memory in humans.

Kofeina osłabia pamięć długotrwałą w teście biernego unikania u myszy

Celem pracy była ocena wpływu jednorazowego podania kofeiny (CAF) na pamięć długotrwałą u myszy poddanych testowi biernego unikania. Wyniki wskazują, iż CAF (podana dootrzewnowo, 30 min., przed testem) w dawkach 69,3 i 92,4 mg/kg istotnie zaburzała pamięć długotrwałą poprzez skrócenie czasu zatrzymania u zwierząt ze 180 s (grupa kontrolna) do odpowiednio 105 s ($p<0.05$) i do 90 s ($p<0.001$). Analiza regresji liniowej ujawniła trend pomiędzy wzrastającymi dawkami CAF a ich wynikającym obniżeniem czasów zatrzymania, wskazując, że CAF upośledzała, w sposób zależny od dawki, pamięć długotrwałą w teście biernego unikania. Na podstawie tego przedklinicznego badania można stwierdzić, że CAF w wysokich dawkach może również upośledzać zapamiętywanie i pamięć u ludzi.