ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXI, N 2, 140 SECTIO D 2006

Chair and Department of Pathophysiology, Chair and Department of General Chemistry
Medical University of Lublin

MAGDALENA CHROŚCIŃSKA-KRAWCZYK,
MAŁGORZATA JARGIEŁŁO, ANNA KĘDRA,
KAMILA FURMANEK-KARWOWSKA, KAROLINA STĘPIEŃ,
KATARZYNA SAWICKA, SEBASTIAN KRAWCZYK

Influence of caffeine alone or combined with antiepileptic drugs (lamotrigine, oxcarbazepine, and tiagabine) on locomotor activity in mice

Caffeine, a member of a class of compounds known as methylxanthines, is widely consumed by humans in a variety of foods, beverages and over-the-counter medicines. Subjective effects, often reported following the consumption of caffeine, include increased energy, alertness and concentration, yet, little is known about the effects of chronic caffeine intake (14). In rodents, acute administration of caffeine produces reliable and concentration-dependent locomotor stimulation, and chronic administration results in tolerance to this effect.

Caffeine (1,3,7-methylxantine), belongs to alkaloids, one of the most frequent nutrients in the world. Most commonly, it is an ingredient of popular beverages like coffee, tea or cola. Also, chocolate products, coconut chips or even analgesics and appetizers contain caffeine (1). In many experiments it has been found that caffeine and other methylxantines suppress anticonvulsive activity of conventional antiepileptic drugs (AEDs) in animal models of epilepsy. The effect has been noted after single and multiple dosages of methylxantines (5, 8). Although there is a high correlation between the acute behavioral stimulant effects of caffeine and other methylxantines and their ability to antagonize adenosine receptors (12), little is known about the mechanisms contributing to the development of tolerance to behavioral stimulant effects of caffeine.

Oxcarbazepine (OXC) belongs to novel antiepileptics. Its mechanism of action is not yet known in details. It is suspected to block sodium channels (9). OXC is licensed mainly for use as monotherapy or adjunctive therapy in patients with partial onset seizures with or without secondary generalization. It has a significant effect both in partial and in secondarily generalized seizures (9).

Tiagabine (TGB), another novel AED, blocks the GABA transporter – GAT1, GAT2, GAT3 and enhances GABA receptor activity by potently and specifically inhibiting the uptake of GABA into neurons and glia (6). Consequently, it leads to an increase of extracellular GABA concentration in the synaptic cleft and to the improvement of GABA neurotrasmission in the brain, which results in reduction of seizure frequency. TGB turned out to be effective in partial seizures and secondly generalized seizures (15).

Lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, LTG), the third novel AED tested, blocks sodium channels, which is probably responsible for the suppression of presynaptic

release of stimulatory amino-acids (3). The drug has been mainly used in the monotherapy of partial epileptic attacks, generalized epileptic seizures (also primary and secondary generalized tonic-clonic seizures) and unconsciousness attacks (3).

As it was mentioned before, caffeine reduced the anticonvulsive activity of AEDs. The caffeine's influence on adverse effects of LTG, TGB and OXC as regards possible locomotor activity disorders has been the main aim of this study.

MATERIAL AND METHODS

The experiments were conducted on female Swiss mice, weighing 22–27 g after the adaptation period of 10 days. Experimental groups, consisting of 8–10 animals, were chosen randomly. The animals were housed in colony cages, under standard laboratory conditions, with unlimited access to food and tap water. All mice were maintained at an ambient temperature of 20 ± 1° and on a natural light-dark cycle. Animals from appropriate groups were tested on the same day, in order to provide optimally objective results in the experiment. Locomotor activity was performed between 8.00 a.m. and 3.00 p.m. and each mouse was used only once. The tested drugs and caffeine were administered intraperitoneally (i.p.) in a volume of 10 ml/kg of body weight. The following antiepileptics were used in this study: lamotrigine (LTG; Lamictal, Glaxo Wellcome, Kent, Great Britain), tiagabine (TGB; Gabitril, Sanofi Winthrop, Gentilly, France) and oxcarbazepine (OXC, Trileptal, Novartis Pharma, Basel, Switzerland). All drugs were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, Mo., USA). Caffeine (Coffeinum natrium benzoicum, Pliva, Kraków, Poland) was available in a sterile saline solution and given i.p. in doses referring to the pure methylxanthine.

The animals were injected twice a day (7.00 a.m. and 7.00 p.m) as follows: Group 1 (control – saline for 14 days twice daily, the last injection on day 15), Group 2, saline for 14 days twice daily and then, on day 15, caffeine in a single dose (acute caffeine), Group 3, caffeine for 14 days, twice daily (chronic caffeine), the last injection being given on day 15. On day 15, mice from all groups received one of the AEDs in a single dose: LTG given 60 min before test at the following doses: 7.6 and 8.2 mg/kg, TGB – 15 min at the dose of 4 mg/kg, and OXC – 30 min before test at the following doses: 14.5 and 15.3 mg/kg. Caffeine was administered 30 min before test; the control group received saline instead of caffeine. Doses of caffeine were 46.2 mg/kg. Caffeine doses and doses of AEDs used in this study were estimated on the basis of the previous research. Briefly, doses of LTG and OXC are their ED_{50} values when combined with caffeine in the maximal electroshock test. The dose of TGB was chosen on the basis of its activity on the threshold for electroconvulsions (4, 8).

Locomotor activity of mice was registered with DIGISCAN Optical Animal Activity Monitoring System (OMNITECH, Columbus, Ohio, USA). The activity chamber was a transparent plastic cage (40 x 40 x 40 cm), equipped with four infrared-activated photocells connected to an electronic event recorder. Locomotor activity in animals (including control group of animals) was estimated after administration of AEDs separately and also in combination with caffeine. Moreover, the analysis of locomotor activity included: the total distance covered by an animal and measurements of motility stereotypes in animals that may happen after AEDs administration within 30 min of observation period. Within the first 15 min, cognitive motility was measured, the next 15 min referred to spontaneous motility. However, for specific behavioral measures, the 30-min session was divided into five 6-min intervals with each mouse being observed for 5 min in every 6-min interval.

The results of locomotor activity of animals were presented in mean values +/- SE. One-way ANOVA followed by Dunnett test was used for statistical analysis. Results were considered significant if the P value was lower than 0.05.

RESULTS

THE EFFECT OF LAMOTRIGINE (LTG) AND CAFFEINE ON LOCOMOTOR ACTIVITY IN MICE

Caffeine (46.2 mg/kg) administered as a single dose and given daily for 14 days, did not influence the total distance and motility stereotypes in animals. LTG given at the doses 7.6 and 8.2 mg/kg did not affect the motility activity in mice. Caffeine (46.2 mg/kg), both acutely and chronically, did not affect the locomotor activity observed after administration of LTG (Table 1).

Table 1. The influence of LTG combined with caffeine on animals locomotor activity in the range of the total distance covered by the animal and motility stereotypes

Total distance				
Acute treatment				
Substances (mg/kg)	0–15 min		15–30 min	
	middle distance (cm)	standard error	middle distance (cm)	standard error
Control	1571.6	264.5	693.5	121.2
LTG 1	1204.3	192	357.2	74.6
Caffeine1	2466.4	347.4	2212	325.3
Mix 1	1256.1	263	703.5	201
Chronic treatment				
Control	1571.6	264.5	693.5	121.2
LTG 2	1335.3	271	472.3	108.5
Caffeine 2	1927.1	144.6	1250.1	170.3
Mix 2	1130.7	124	495.5	46.5
Motility stereotypes				
Acute treatment				
Substances (mg/kg)	0–15 min		15–30 min	
	middle numbers of motility stereotypes	standard error	middle numbers of motility stereotypes	standard error
Control	652	75	385.3	50.2
LTG 1	530.2	63.5	202.8	48.1
Caffeine1	627.2	88	641.2	73.8
Mix 1	356.5	108.7	288.6	102.4
Chronic treatment				
Control	652	75	385.3	50.2
LTG 2	467.8	67.5	208.3	44
Caffeine 2	647.1	69	531.3	89
Mix 2	299.1	42.1	164.5	51

Control – 0.9% NaCl, caffeine 1 – acute caffeine, LTG 1 – 8.2 mg/kg, Mix 1 – caffeine and LTG 1, caffeine 2 – chronic caffeine, LTG 2 – 7.6 mg/kg, Mix 2 – caffeine and LTG 2

THE EFFECT OF TIAGABINE (TGB) AND CAFFEINE ON THE LOCOMOTOR ACTIVITY IN MICE

Caffeine (46.2 mg/kg) administered as a single dose and given daily for 14 days, did not influence the total distance and motility stereotypes in animals. TGB, given at 4 mg/kg, did not affect the total distance covered by the animals. However, TGB decreased the motility stereotypes in mice. Caffeine (46.2 mg/kg) administered as a single dose and daily for 14 days, did not affect the locomotor activity (Table 2).

Table 2. The influence of TGB combined with caffeine on animals locomotor activity in the range of the total distance covered by the animal and motility stereotypes

Total distance				
Acute treatment				
Substances (mg/kg)	0–15 min		15–30 min	
	middle distance (cm)	std. err.	middle distance (cm)	std. err.
Control	1413.5	204	1044.5	232
TGB	1152	325	574.5	216
Caffeine 1	2401.7	385	1747.6	418.5
Mix 1	1257.8	132.4	477.7	90.4
Chronic treatment				
Control	1413.5	204	1044.5	232
TGB	1152	325	574.5	683
Caffeine 2	2124.2	194.4	1759.3	224.8
Mix 2	1328.8	278.2	1020.5	288.3
Motility stereotypes				
Acute treatment				
Substances (mg/kg)	0–15 min		15–30 min	
	middle numbers of motility stereotypes	std. err.	middle numbers of motility stereotypes	std. err.
Control	548.7	47.8	479.2	60.9
TGB	150.1 **	54.3	44.7 ***	29.3
Caffeine 1	609	91.3	509	93.7
Mix 1	234.8 *	64.5	65.7 ***	31
Chronic treatment				
Control	548.7	47.8	479.2	60.9
TGB	150.1 **	54.3	44.7 ***	29.3
Caffeine 2	534.6	44.5	494.6	72.4
Mix 2	82 **	20	20.8 ***	8.8

Control – 0.9% NaCl, caffeine1 – acute caffeine, TGB - 4 mg/kg, Mix 1 – caffeine and TGB, caffeine 2 – chronic caffeine, Mix 2 – caffeine and TGB

THE EFFECT OF OXCARBAZEPINE (OXC) ON THE LOCOMOTOR ACTIVITY IN MICE

Single and 14-day- administration caffeine (46.2 mg/kg) did not affect the total distance covered by the animal and the number of motility stereotypes. OXC, given at the doses 14.5 and 15.3 mg/kg, did not influence the locomotor activity in animals. Caffeine (46.2 mg/kg) used both as a single dose and given daily for 14 days, did not affect the OXC activity (Table 3).

Table 3. The influence of OXC combined with caffeine on animals locomotor activity in the range of the total distance covered by the animal and motility stereotypes

Total distance				
Acute treatment				
Substances (mg/kg)	0–15 min		15–30 min	
	middle	std. err.	middle	std. err.
Control	745	113	588	101.3
OXC 1	691.8	112.6	543.1	113.5
Caffeine 1	920.6	98.2	961.8	112.1
Mix 1	935.6	100	840.6	108.6
Chronic treatment				
Control	745	113	588	101.3
OXC 2	399.1	111.1	267.7	29.2
Caffeine 2	925.7	96.5	741	121.6
Mix 2	589.2	104	533	111.7
Motility stereotypes				
Acute treatment				
Substances (mg/kg)	0–15 min		15–30 min	
	middle	std. err.	middle	std. err.
Control	337.5	35.3	329.8	43.1
OXC 1	318.2	53	203.3	45.1
Caffeine 1	613	19.6	560.5	45.3
Mix 1	393.2	55.4	382.1	67
Chronic treatment				
Control	337.5	35.3	329.8	43.1
OXC 2	126.1	34.6	94.5	15.3
Caffeine 2	737	56.6	796.1	32.7
Mix 2	708.2	42.1	753.3	30.4

Control – 0.9% NaCl, caffeine 1 – acute treatment, OXC 1 – 15.3 mg/kg; Mix 1 – caffeine 1 and OXC 1, caffeine 2 – chronic treatment, OXC 2 – 14.5 mg/kg, Mix 2 – caffeine 2 and OXC 2

DISCUSSION

It is noteworthy that caffeine and aminophylline (theophylline$_2$ ethylenediamine), another methylxanthine derivative, reduce the anticonvulsant effects of many AEDs, including conventional and novel ones in models of electrically-, amygdala-kindled- or pentetrazole-induced seizures (5, 8). Interestingly, chronic caffeine retains this dangerous potential which is even potentiated over time towards AEDs (8). Proconvulsive activity of caffeine is probably associated with its antagonism of adenosine receptors. It relates mainly to A_1 and A_2 receptors, but less so to A_3 (7). However, the hazardous potential of methylxanthines towards AEDs is not likely to result from the blockade of adenosine receptors in the central nervous system (CNS) (5). The data from experimental models of epilepsy show that this potential may be associated with a methylxantine produced increased release of calcium ions from endoplamic reticulum and intraneuronal calcium accumulation. Another possibility assumes the enhanced release of excitatory amino acids (5).

Also, some clinical reports have revealed that caffeine reduces the protective activity of conventional AEDs in epileptic patients (2), which points to a good correlation between the experimental and clinical data.

Caffeine is the most often used psychostimulant. The dose necessary for eliciting significant stimulatory effects is about 200 mg daily. It is known that worldwide caffeine is consumed in different forms by 90% of population (13).

The caffeine's influence on locomotion in the presence of LTG, TGB and OXC has been taken into consideration and evaluated in this work. Available data differ considering caffeine's influence on this parameter. Kayir and Uzbay (11) showed the stimulating effect of caffeine (0.25–128 mg/kg) on animals' locomotor activity. Kaplan et al. (10) found in their study that caffeine exerted both stimulating and inhibitive influence on motility stereotypes of mice. The methylxanthine given acutely (97–194 mg/kg) stimulated animals' locomotor activity but inhibited this parameter after prolonged administration.

The results of this study show that caffeine, LTG and OXC, given alone, have no influence on locomotor activity in mice. Only TGB reduced the number of motility stereotypes. Interestingly, caffeine combined with AEDs, did not modify their effects on locomotor activity, although a tendency to further reduce TGB-induced stereotypes was observed.

CONCLUSIONS

Caffeine administered acutely and chronically, did not influence the total distance and motility stereotypes in animals. LTG and OXC did not affect the locomotor activity in mice. TGB reduced only the motility stereotypes in mice. It did not affect the total distance covered by an animal. Combinations of caffeine with AEDs did not further induce their effects upon locomotion. This indicates that apart from a clear-cut reduction of the anticonvulsant effects of AEDs, caffeine is not likely to affect their adverse potential.

REFERENCES

1. Bernstein G. A. et al.: Caffeine dependence in teenagers. Drug and Alcohol Dependence, 66, 1, 2002.
2. Bonhilha L., Li M.: Heavy coffee drinking and epilepsy. Seizure,13, 284, 2004.
3. Borowicz K. K. et al.: Influence of several convulsants on the protective activity of a

non-competitive AMPA/ kainate antagonist, LY 300164, and lamotrigine against maximal electroshock in mice. J. Physiol. and Pharmacol., 53, 859, 2002.
4. Chrościńska-Krawczyk M.: Wpływ kofeiny na efekty ochronne nowych leków przeciwpadaczkowych – lamotryginy, tiagabiny i okskarbazepiny w drgawkach elektrycznych u myszy. Praca doktorska, AM Lublin, 2005.
5. Czuczwar S. J., Kleinrok Z.: Modulation of the protective efficacy of common antiepileptic drugs by xanthine derivatives: implications for the clinical use of xanthines in epileptic patients. Pharmacol. Res., 22, 661, 1990.
6. Czuczwar S. J., Patsalos P.: The new generation of GABA enhancers. Potential in the treatment of epilepsy. CNS Drugs, 15, 339, 2001.
7. Dingledine R. et al.: Excitatory amino acid receptors in epilepsy. TIPS Special Report, 49, 1991.
8. Gąsior 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.
9. Kalis M. M., Huff N. A.: Oxcarbazepine, an antiepileptic agents. Clin. Ther., 23, 680, 2001.
10. Kaplan G. B. et al.: Caffeine treatment and withdrawal in mice: relationships between dosage, concentrations, locomotor activity and A_1 adenosine receptor binding. J. Pharmacol. Exp. Ther., 266, 1563, 1996.
11. Kayir H., Uzbay I. T.: Evidence for the role of nitric oxide in caffeine-induced locomotor activity in mice. Psychopharmacology, 172, 11, 2004.
12. Kuzmin A.: Differences in the effect of chronic and acute caffeine on self-administration of cocaine in mice. Eur. J. Neurosci., 12, 3026, 2000.
13. Nehlig A. et al.: Caffeine and the central nervous system: Mechanism of action, biochemical, metabolic and psychostimulant effects. Brain Res. Rev., 17, 139, 1992.
14. Powell K. R. et al.: The role of dopamine in the locomotor stimulant effects and tolerance to these effects of caffeine. Pharmacol. Biochem. Behav., 69, 59, 2001.
15. Schmidt D. et al.: Tiagabine in the treatment of epilepsy – a clinical review with a guide for the prescribing physician. Epilepsy Research, 41, 245, 2000.

SUMMARY

The influence of lamotrigine (LTG, 60 min before testing), oxcarbazepine (30 min before testing), tiagabine (TGB, 15 min before testing), alone or combined with caffeine (a single dose 46.2 mg/kg – 30 min before testing – or 14-day administration twice daily at 7.00 a.m. and 7.00 p.m) on locomotor activity in mice was evaluated. All drugs and caffeine were given intraperitoneally (i.p.). TGB was given at doses: 7.6 and 8.2 mg/kg, TGB at the dose of 4 mg/kg, and OXC at the doses: 14.5 and 15.3 mg/kg. The total distance covered by an animal and a number of motility stereotypes were estimated. Locomotor activity of mice was registered with DIGISCAN Optical Animal Activity Monitoring System (OMNITECH, Columbus, Ohio, USA). No effect of caffeine, lamotrigine and oxcarbazepine on locomotor activity was found. Tiagabine was the only drug that affected the locomotor activity by decreasing the number of motility stereotypes. Also, no effect of caffeine on motor activity disturbances caused by tiagabine was found. Combinations of caffeine with lamotrigine or oxcarbazepine were without effect on locomotor activity.

Wpływ kofeiny, zastosowanej samej lub w kombinacji z lekami przeciwpadaczkowymi (lamotryginą, okskarbazepiną i tiagabiną) na aktywność motoryczną myszy

W pracy badano wpływ nowych leków przeciwpadaczkowych: lamotryginy (LTG), okskarbazepiny (OXC) i tiagabiny (TGB), stosowanych pojedynczo lub w kombinacji z kofeiną, na aktywność motoryczną myszy. Wszystkie leki oraz kofeina podawane były dootrzewnowo. LTG podawano 60 min. w dawkach 7,6 i 8,2 mg/kg, TGB 15 min.w dawce 4 mg/kg, a OXC 30 min. przed testem w dawkach 14,5 i 15,3 mg/kg. Kofeinę, w dawce 46,2 mg/kg, stosowano jednorazowo 30 min. przed testem oraz przewlekle, podając ją zwierzętom dwa razy dziennie przez 14 dni. Pomiarów dokonywano przy pomocy komputerowego miernika ruchliwości DIGISCAN (OMNITECH, Columbus, Ohio, USA). Na aktywność motoryczną myszy składały się: całkowity dystans przebyty przez zwierzę oraz liczba stereotypii ruchowych. Na podstawie przeprowadzonych doświadczeń nie stwierdzono wpływu kofeiny, LTG oraz OXC na aktywność motoryczną zwierząt. TGB była jedynym lekiem, który wpływał na badany parametr poprzez zmniejszenie liczby stereotypii ruchowych. Nie stwierdzono jednak wpływu kofeiny na powyższe zaburzenia motoryki wywołane przez TGB. Łączne zastosowanie kofeiny z lamotryginą i okskarbazepiną nie wpłynęło na aktywność motoryczną myszy.