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Formation of stacking complexes between caffeine (1,2,3-trimethylxanthine) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine may attenuate biological effects of this neurotoxin

Katarzyna Ulanowska [a], Jacek Piosik [b], Anna Gwizdek-Wiśniewska [c], Grzegorz Wegrzyn [a,d,*]

[a] *Department of Molecular Biology, University of Gdańsk, Kładki 24, 80-822 Gdansk, Poland*
[b] *Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Kładki 24, 80-822 Gdansk, Poland*
[c] *Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Narutowicza 11, 80-952 Gdansk, Poland*
[d] *Institute of Oceanology, Polish Academy of Sciences, Św. Wojciecha 5, 81-347 Gdynia, Poland*

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Abstract

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin causing symptoms that may resemble those observed in patients suffering from Parkinson's disease. Therefore, MPTP-treated laboratory animals are currently the most favored models to study therapeutic intervention strategies in this disease. It was demonstrated recently that caffeine (1,2,3-trimethylxanthine) intake decreases the risk of Parkinson's disease in various human populations and attenuates MPTP-induced neurological effects in animal models. Since the effects of caffeine on MPTP-treated animals were mimicked by several antagonists of the adenosine A_{2A} receptor, it was suggested that caffeine attenuates MPTP toxicity by blocking this receptor. Here, using microcalorimetry and molecular modeling, we demonstrate that caffeine can form stacking (π–π) complexes with MPTP. We found that a biological activity of MPTP (induction of

* Corresponding author. Fax: +48 58 301 0072.
E-mail address: wegrzyn@biotech.univ.gda.pl (G. Wegrzyn).

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mutations in a microbiological mutagenicity assay), which is completely independent on the A_{2A} receptor blockade, is significantly reduced by caffeine. Therefore, we suggest that caffeine may attenuate neurotoxicity of MPTP (and possibly other polycyclic aromatic toxins) and reveal its protective effects on the risk of Parkinson's disease not only by blocking the A_{2A} receptor but also by sequestering neurotoxin molecules in mixed complexes, especially in stomach.

Keywords: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 1,2,3-trimethylxanthine; Parkinson's disease; Stacking complexes; Mutagenicity

1. Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Fig. 1A) is a neurotoxin, which induces in humans a syndrome virtually identical to Parkinson's disease and is extensively used in studies on animal models of this disorder (for recent reviews about MPTP and Parkinson's disease see [1–10]). Parkinson's disease is characterized by massive degeneration of dopaminergic neurons in the substantia nigra, the loss of striatal dopaminergic fibers and a dramatic reduction of the striatal dopamine levels. The massive striatal dopamine deficiency causes motor deficits in patients, including bradykinesia, rigidity, and resting tremor, which are the most characteristic symptoms of this disease.

MPTP selectively damages dopaminergic neurons, predominantly those originating in the substantia nigra pars compacta. This leads to impaired dopaminergic neurotransmission accompanied by a loss of dopaminergic nerve terminals in the striatum. Primates treated with MPTP develop motor disturbances resembling those observed in patients suffering from Parkinson's disease, namely bradykinesia, rigidity, and postural abnormalities. Therefore, MPTP-based animal models have played a crucial role in elucidating the mechanism of Parkinson's disease. Importantly, MPTP-treated animals (particularly primates) are responsive to all commonly used antiparkinsonian agents, and effects of these agents assessed in such studies have proved to be highly predictive of symptomatic drug action in Parkinson's disease. In addition, compounds antagonizing or potentiating MPTP effects have indicated new therapies for this disease [1–10].

Epidemiological studies indicated that higher coffee intake is associated with a significantly lower incidence of Parkinson's disease [11]. Results of those studies suggested that the mechanism of this phenomenon may be related to caffeine intake and not to other nutrients contained in coffee. Subsequently, a protective effect of moderate doses of caffeine on risk of Parkinson's disease in various human populations was confirmed [12,13]. Moreover, it was demonstrated that caffeine attenuated MPTP-induced loss of striatal dopamine and dopamine transporter binding sites [14,15]. In accordance to these findings, it was reported that caffeine improves the memory deficits observed in MPTP-lesioned rats [16].

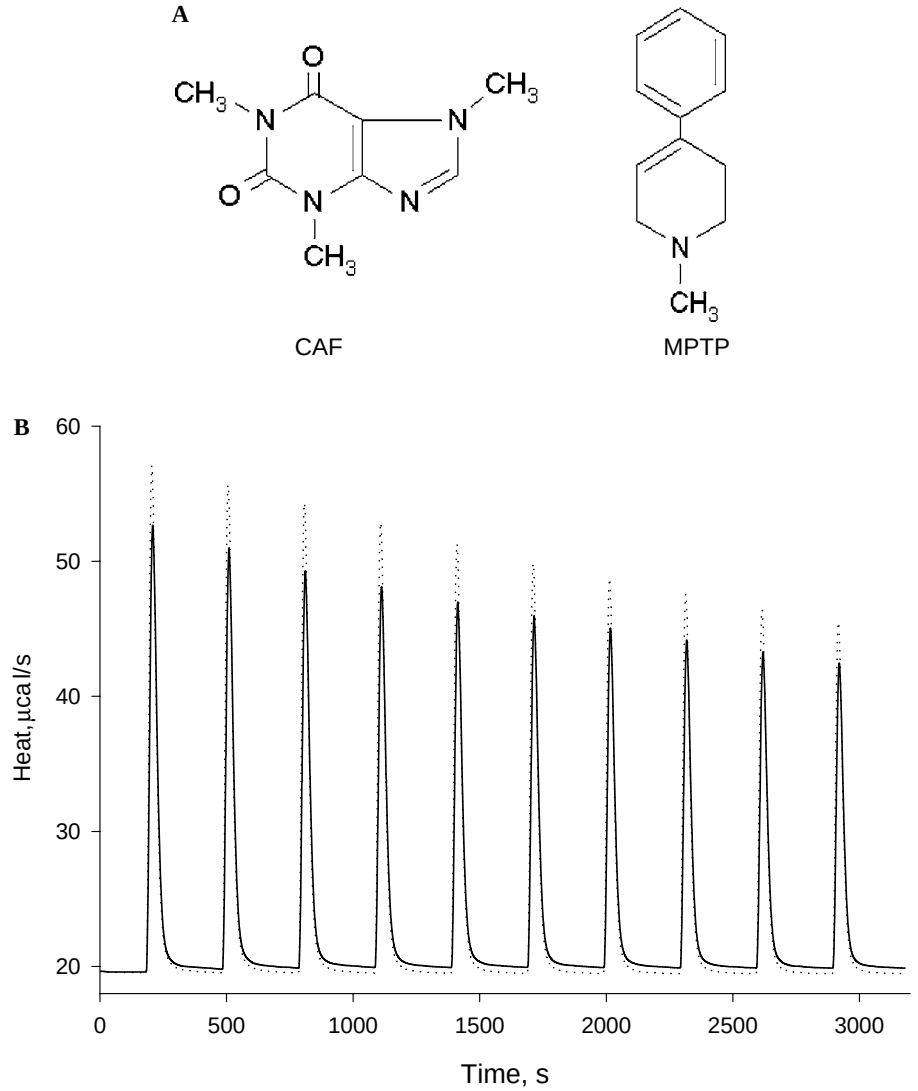

Fig. 1. Chemical structures of caffeine (CAF; 1,2,3-trimethylxanthine) and MPTP (1-methy-4-phenyl-1,2,3,6-tetrahydropyridine) (A) and titration of MPTP with caffeine (B). (B) Microcalorimetric titration of MPTP (initial concentration of 1.28 mM) with caffeine (CAF) (stock solution concentration 101.85 mM) (solid line), and buffer with caffeine (dotted line). Ten aliquots of caffeine (10 µL each) were added at 5 min intervals and the heat of exchange (in µcal/s) was measured as a function of time.

Caffeine (1,2,3-trimethylxanthine; Fig. 1A), apart from its other activities, is an antagonist of the adenosine A_{2A} receptor [17]. Since the effects of caffeine on MPTP-treated animals were mimicked by several A_{2A} antagonists [14,18,19] and by genetic inactivation of the A_{2A} receptor [14], it was suggested that caffeine attenuates MPTP toxicity by A_{2A} receptor blockade [14,20].

It was demonstrated recently that caffeine can form stacking (π–π) complexes with various polycyclic aromatic agents [21–24]. Formation of mixed aggregates with caffeine might reduce concentrations of aromatic agents in their free forms in solution, preventing their biological activities. Since MPTP is an aromatic chemical (Fig. 1A), here we asked whether caffeine can form stacking complexes with this compound and reduce its biological activity by an additional mechanism, apart from already postulated [14,20] blocking of the A_{2A} receptor.

2. Methods

2.1. Chemicals

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) and caffeine (1,2,3-trimethylxanthine) were used. These chemicals were purchased from Sigma–Aldrich.

2.2. Microcalorimetry

The microcalorimetric titrations were performed at 30 ± 0.1 °C in the buffer composed of 5 mM Hepes and 0.15 M NaCl, pH 7.4, using Omega Titration Calorimeter (Microcal). Then, portions of caffeine (stock solution concentration 101.9 mM), 10 μL each, were added to 1.3 mL of the titrated solution and the heat process was measured as a function of time (μcal/s). The results of titration were corrected for heat of components' dilution, and expressed as the heat per mol of caffeine, as described previously [21–23].

2.3. Molecular modeling

All the calculations were performed using HyperChem software (Hypercube). The semi-empirical PM3 method with steepest descent algorithm for geometry optimization and minimization of total energy of the system was used. Molecules of caffeine, MPTP and their several possible configurations in complexes were built and optimized (terminal condition—RMS gradient lower than 0.2 for molecules alone or 0.3 for complexes). On the basis of these calculations, optimal geometries of the complexes have been evaluated. The energy of MPTP–caffeine complex formation was estimated by subtraction of energies of formation of caffeine and MPTP (both molecules in the same conformation as in the complex) from total energy of the complex.

2.4. The Vibrio harveyi mutagenicity assay

The *V. harveyi* mutagenicity assay is based on the use of a series of genetically modified strains of this bacterium [25–27]. *V. harveyi* is naturally sensitive to neomycin, but mutants resistant to this antibiotic can be isolated. The frequency of the

appearance of such mutants increases in the presence of mutagens in a dose–response manner [25,26]. To assess mutagenicity of MPTP in the presence or absence of caffeine, *V. harveyi* BB7 [28] and BB7M [25] strains (as various *V. harveyi* tester strains respond differentially to different mutagens, in preliminary experiments we determined that BB7 and BB7M are the most sensitive strains to MPTP mutagenicity) were cultivated in the BOSS medium [29] at 30 °C. To exponentially growing cultures, indicated amounts of MPTP and/or caffeine were added and cultivation was continued for 3 h. Then, 5×10^6 cells were spread onto BOSS agar plates containing neomycin (0.1 mg/mL). Following 48 h incubation at 30 °C, neomycin-resistant colonies were counted. To estimate number of spontaneous neomycin-resistant mutants, analogous experiments were performed in which neither MPTP nor caffeine was added. Number of such mutants was then subtracted from number of mutants in cultures treated with MPTP and/or caffeine.

3. Results

3.1. Microcalorimetric studies on direct interactions between caffeine and MPTP

To study interactions between caffeine and MPTP, we used the method of titration microcalorimetry. The UV/Vis spectroscopy could not be employed in this case because two analyzed compounds have absorption abilities in similar wavelength ranges (maximum of caffeine absorption is at 272 nm and that of MPTP is at 240 nm).

Results of titrations of MPTP (initial concentration 1.28 mM) with caffeine (stock solution concentration 101.85 mM; concentration range in cell 0.75–7.22 mM) and buffer with caffeine are presented in Fig. 1B. The latter titration has been performed to estimate the heat of dilution of caffeine. Control titrations of MPTP with buffer (heat of dilution of MPTP) and buffer with buffer (heat exchange between syringe and cell) have also been carried out (data not shown). The approximate value of heat of interaction between MPTP and caffeine was corrected by subtraction of the sum of heats of dilution of caffeine and MPTP. The obtained results were extrapolated to values of [CAF]/[MPTP] $\rightarrow 0$ (where CAF, caffeine) and are presented in Fig. 2 as kcal/mol of titrant injected (caffeine) vs. [CAF]/[MPTP]. Calculated heat of interaction between MPTP and caffeine is $\Delta H = -149$ (± 2) cal/mol. These results strongly suggest that caffeine can directly interact with MPTP.

3.2. Molecular modeling

MPTP was purchased as a hydrochloride salt which exists in a water solution as a cation ($pK_a = 8.66$ [30]) in four possible conformations. The 1,2,3,6-tetrahydropyridine ring adopts only the envelope conformation because of the appearance of a double bond. The methyl group may appear in an equatorial or axial position. The structures with the methyl group at an axial position are less energetically preferred because of the steric hindrance. There are only two possible conformations with the methyl group in an equatorial position. These two energetically preferred

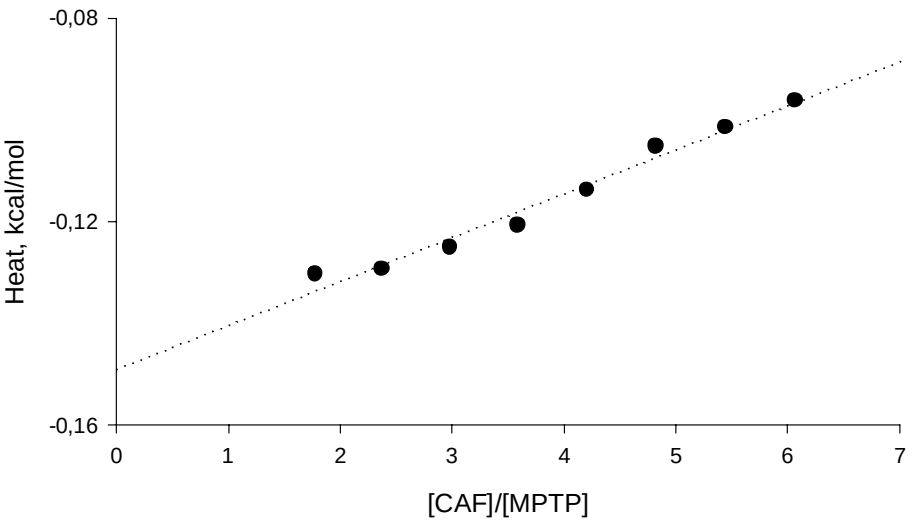

Fig. 2. Heat of interaction between MPTP and caffeine as estimated by microcalorimetric titration. The heat of reaction ($\Delta H = -149$ cal/mol) was corrected for the heat of dilution of components (not shown) and calculated by extrapolation to [CAF]/[MPTP] $\rightarrow$ 0 (CAF, caffeine).

conformations differ only by a position of the double bond (Figs. 3A and B). These structures were used for further calculations.

Results of the molecular modeling (performed as described under Section 2) indicated that MPTP can potentially form stacking complexes with caffeine. The most stable putative complexes are presented in Fig. 3C–H. The distance between rings is equal to about 3.4–3.6 Å, and the hydrogen bond might be formed between the hydrogen connected to nitrogen in MPTP and oxygen at C2 in caffeine. The appearance of intermolecular hydrogen bond may stabilize the stacking complexes. The formation energy of such complexes has been estimated at about −12 kcal/mol.

3.3. Reduction of mutagenic activity of MPTP by caffeine

Since results of our studies described above (Figs. 1–3) strongly suggested that caffeine can form stacking complexes with MPTP, we asked whether these interactions have any biological relevance. One might assume that due to formation of mixed complexes between caffeine and MPTP, a concentration of free molecules of the latter compound should decrease, resulting in its reduced biological activity. We aimed to test this hypothesis, however, since caffeine-mediated attenuation of MPTP neurotoxicity was previously proposed to arise from the A_{2A} receptor blockade [14,20], animal models would not be optimal in such studies due to putative overlapping of these two possible mechanisms. One could consider the use of animals with knock-out mutations in the gene coding for the A_{2A} receptor or the use of blockers of this receptor. However, both experimental systems would result in the attenuated response to MPTP, similar to that caused by caffeine [14,20]. This would potentially

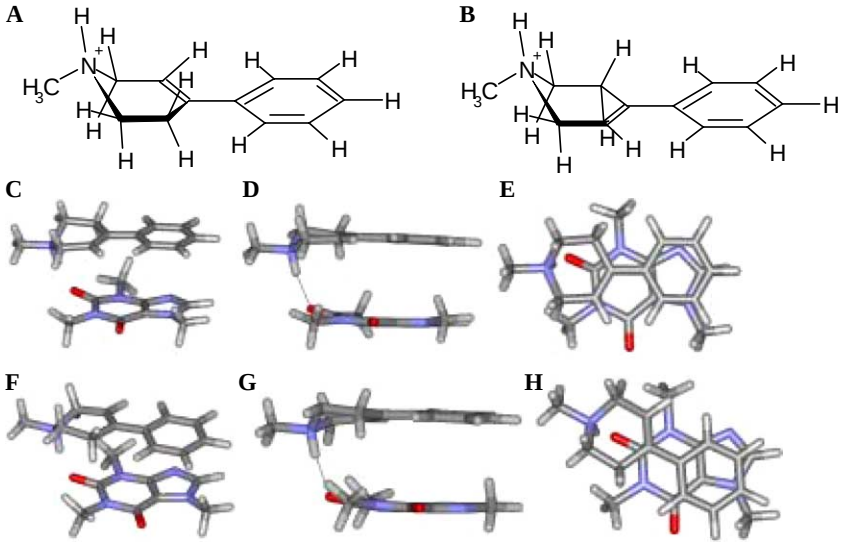

Fig. 3. MPTP conformations revealing the lowest energy (A and B) and formation of possible stacking complexes between MPTP and caffeine, predicted by molecular modeling (C–H). (C and F) MPTP adopts two different conformations (presented in A and B, respectively). Energies of formation of these complexes do not differ significantly (−11.7 and −12.0 kcal/mol). (D, E and G, H) Complexes depicted in (C and F), respectively, from other perspectives. The intermolecular hydrogen bond (shown on D and G as thin lines) stabilizes the complex. For clarity of the figure, double bonds are not shown.

cause serious problems with interpretation of results. Therefore, we decided to measure a biological activity of MPTP which is completely independent on the A_{2A} receptor status. Mutagenicity of MPTP, demonstrated previously [31], fulfills this requirement. Thus, we assessed mutagenic activity of MPTP in the absence and presence of caffeine. We employed the *V. harveyi* mutagenicity assay [25–27], which has been demonstrated previously to be suitable for assessment of mutagenic activities of various chemicals [24,26,32].

We observed a mutagenic activity of MPTP (Fig. 4A), which corroborates previously reported conclusion based on results obtained using another mutagenicity assay [31]. In our assays, caffeine had no considerable mutagenic activity at concentrations up to 1 mM (data not shown). At the MPTP concentration (1.2 μM) giving the highest mutagenicity in the assays with the BB7 tester strain, we observed a significant, dose-dependent reduction of this effect by caffeine (Fig. 4B). Similar results were obtained when another tester strain, BB7M (which is generally more sensitive to various mutagens), was used (Fig. 4C), confirming that the observed phenomenon is not strain-specific. Reduction of MPTP mutagenicity in the presence of caffeine was also observed at MPTP concentrations lower than 1.2 μM (results not shown), indicating that a putative increase in bioavailability of MPTP by caffeine (and resultant decrease in mutagenicity, resembling that observed at 2.4 μM MPTP in the absence of caffeine; see Fig. 4A) is unlikely.

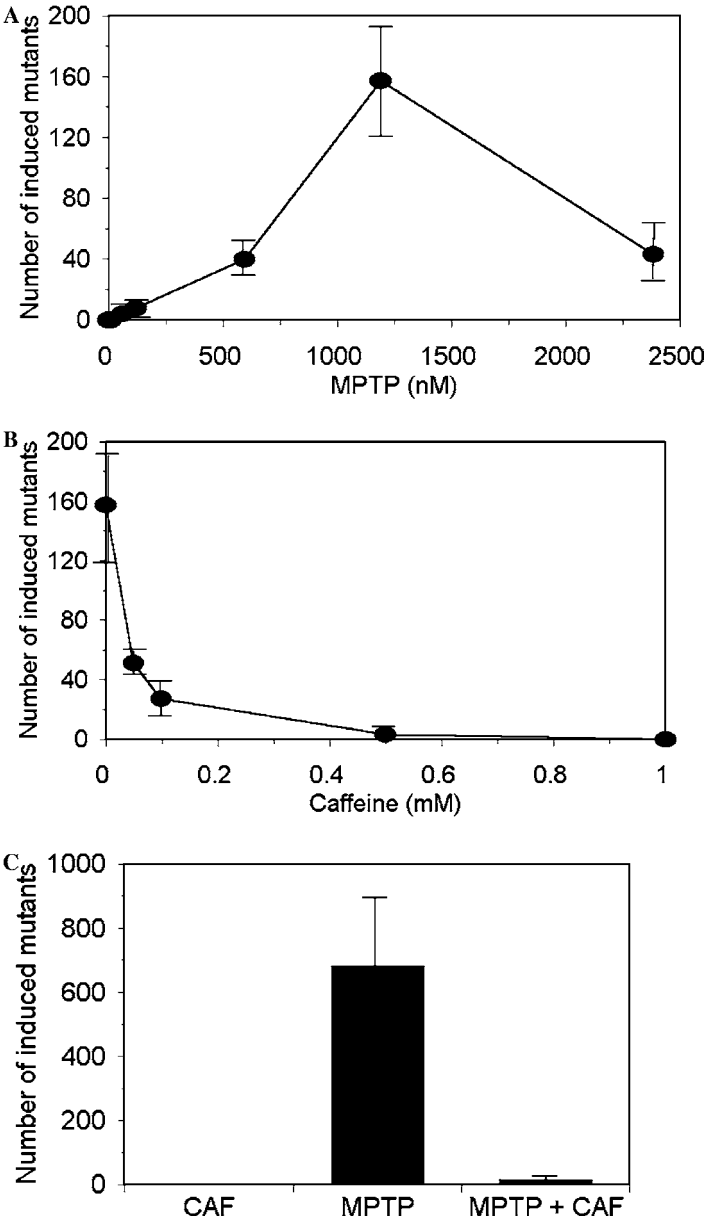

Fig. 4. Mutagenicity of MPTP and its attenuation by caffeine. (A) Mutagenicity of MPTP as assessed using the *V. harveyi* mutagenicity assay. Cultures of the BB7 strain were treated with indicated concentrations of MPTP. (B and C) Effects of caffeine on mutagenicity of MPTP. In experiments shown in (B), cultures of the *V. harveyi* BB7 strain were treated with 1.2 μM MPTP and indicated concentrations of caffeine. In experiments shown in (C), cultures of the *V. harveyi* BB7M strain were treated with either 0.5 mM caffeine (CAF) or 1.2 μM MPTP (MPTP) or both (MPTP + CAF). In all panels, presented results are mean values from 3–5 experiments with bars indicating standard deviation.

4. Discussion

Parkinson's disease is the second most common neurodegenerative disorder, which affects up to a few percent of human population over the age of 65 years [17]. Since treatment of animals with MPTP causes the clinical symptoms that resemble those observed in patients suffering from Parkinson's disease, this neurotoxin has been very useful in studies on mechanisms of this disease, as well as on development of therapeutic intervention strategies [3,4,6,7,10].

It was demonstrated recently that caffeine has a protective effect on risk of Parkinson's disease in various human populations [11–13]. Moreover, caffeine attenuates MPTP-induced neurological effects in mice models [14–16]. Since the effects of caffeine on MPTP-treated animals were mimicked by several antagonists of the adenosine A_{2A} receptor, it was suggested that caffeine attenuates MPTP toxicity by blocking this receptor[14,20].

Here we present results strongly suggesting that caffeine interacts with MPTP directly. Heat of this interaction, estimated by microcalorimetric titration, is $\Delta H = -149\ (\pm 2)$ cal/mol. This value is similar to that obtained for caffeine–quinacrine mustard interaction ($\Delta H = -116$ cal/mol) [22]. Formation of mixed aggregates between caffeine and quinacrine mustard has been confirmed by UV/Vis spectroscopy and by statistical–thermodynamical model of mixed aggregation, with mixed association constant of the interaction $K_{AC} = 59.1\ (\pm 2)\ M^{-1}$ [22]. Similar experiments were performed to demonstrate caffeine interactions with other compounds, like fluorochromes [21,33,34], selected anticancer drugs [23] and some mutagens [22,24]. All these compounds have aromatic structures and form stacking complexes with caffeine. These interactions have been confirmed by NMR studies [35–37]. Importantly, caffeine does not interact with mutagens of different (non-aromatic) chemical structures and does not reduce their mutagenicity [22,24]. MPTP molecule has an aromatic structure and potentially can form π–π complexes with caffeine, as demonstrated in this report by molecular modeling and microcalorimetry. Such interactions might lower the concentrations of a free, active form of this neurotoxin.

We found that addition of caffeine attenuates biological effects of MPTP in an experimental system that is completely independent on activity of the A_{2A} receptor, i.e., in the *V. harveyi* mutagenicity assay. Using this assay, we detected a mutagenic effect of MPTP similar to that reported previously [31], though we observed a maximal mutagenicity at several-fold lower than the toxin concentrations. This may be explained by a higher sensitivity of the *V. harveyi* mutagenicity assay in comparison to the Ames test (as demonstrated for some other mutagens [26]), which was employed previously [31]. Nevertheless, in both experimental systems, a decrease in the number of detected mutants was observed at high MPTP concentrations. This might arise from induction of multiple mutations in cells, causing lethal effects or drastic decrease of growth rate, which prevented formation of bacterial colonies.

Significant (more than 3-fold) inhibition of MPTP-mediated induction of mutations in the *V. harveyi* assay was observed at molar ratio of caffeine to MPTP equal to ~40:1. Complete (or almost complete) biological inactivation of the toxin was achieved at the molar ratios between 400:1 and 800:1. These values might appear

relatively high, however, it is worth noting that in studies on animal models of Parkinson's disease, commonly used concentrations of MPTP are between 1.73 μM (equal to 0.3 mg/kg) in monkeys and 173 μM (equal to 30 mg/kg) in mice [38], and concentrations of caffeine used in the animal studies were around 100 μM (equal to about 20 mg/kg). This may correspond to caffeine intake experienced by coffee drinkers that use a few cups of coffee daily, as average caffeine content per a 16-oz cup of coffee was estimated to about 200 mg [39]. Therefore, in some studies on caffeine-mediated attenuation of MPTP neurotoxicity, amounts of caffeine might be sufficiently high to sequester a part of the neurotoxin molecules.

Apart from laboratory studies on MPTP models, even more pronounced effects of neurotoxin sequestration by caffeine could be expected in the protection of humans against Parkinson's disease. Namely, man-made toxins, such as industrial chemicals and herbicides or pesticides, have been suggested to increase the risk of developing of Parkinson's disease. The significance of such factors in etiology of this disease has recently been highlighted [1]. Many of the toxins mentioned above are aromatic chemicals. Since caffeine was found to be able to form stacking complexes with various polycyclic aromatic agents [21–24], including a neurotoxin (this report), one might assume that mixed complexes can also be formed between caffeine and some other neurotoxins that may be involved in development of parkinsonism. In fact, analysis of structures of molecules that were demonstrated to act as neurotoxins causing Parkinson's disease, like paraquat [40–43], salsolinol [44], 6-hydroxydopamine [45] or methamphetamine [46], strongly suggest that due to their aromatic character they can form stacking complexes with caffeine (our unpublished data).

It is worth noting that toxins occur in natural environment usually at very low concentrations, and effects of these chemicals are observed due to their long-term accumulation in organisms. Thus, concentrations of caffeine in bodies of persons that use this compound in their diet might be high enough to sequester a large portion of polycyclic aromatic toxins. Moreover, we would like to stress that concentrations of caffeine, when administered orally, are especially high in a digestive tract. Thus, we suspect that the protective action of caffeine, due to formation of complexes with neurotoxins, might be significant in stomach, and to some extend in intestine, after oral administration of this compound (either with coffee or as a purified chemical), rather than in blood or brain where it would be diluted. Therefore, previously reported results [14], indicating little influence of caffeine on levels of MPTP derivatives in mice brain, are not contradictory to our proposal. Namely, in those experiments both MPTP and caffeine were administered by an intraperitoneal injection, and relatively high concentrations (10–40 mg/kg) of both compounds were used, whereas caffeine can effectively form stacking complexes with MPTP when present at concentrations many times higher than those of the neurotoxin. Such a proportion of these two compounds may occur in stomach but not in experiments with intraperitoneal injections, discussed above.

In summary, we suggest that caffeine may attenuate neurotoxicity of MPTP (and possibly other polycyclic aromatic toxins) and reveal its protective effects on the risk of Parkinson's disease not only by blocking the A_{2A} receptor but also by sequestering neurotoxin molecules in mixed complexes, especially in stomach. Interestingly,

A_{2A} receptor antagonists that can mimic effects of caffeine on MPTP-treated animals [14,18,19], e.g., SCH58261, 3,7-dimethyl-1-propargylxanthine, KW-6002 or KF18446, are polycyclic aromatic agents that may also potentially form stacking complexes (our unpublished data). Moreover, a structure of MPP^+ molecule, which is actually the neurotoxic species that mediates MPTP neurotoxicity [3], indicates that it can form stacking complexes with caffeine, while it is unlikely that caffeine may influence a conversion of MPTP to MPP^+ (our unpublished results).

Acknowledgments

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References

[1] T. Fukuda, Neuropathology 21 (2001) 323–332.
[2] N. Schmidt, B. Ferger, J. Neural. Transm. 108 (2001) 1263–1282.
[3] S.G. Speciale, Neurotoxicol. Teratol. 24 (2002) 607–620.
[4] W. Dauer, S. Przedborski, Neuron 39 (2003) 889–909.
[5] O. Eberhardt, J.B. Schulz, Toxicol. Lett. 139 (2003) 135–151.
[6] P. Jenner, Parkinsonism Relat. Disord. 9 (2003) 131–137.
[7] S. Przedborski, M. Vila, Ann. N.Y. Acad. Sci. 991 (2003) 189–198.
[8] P.J. Blanchet, F. Calon, M. Morissette, A.H. Tahar, N. Belanger, P. Samadi, R. Grondin, L. Gregoire, L. Meltzer, T. Di Paolo, P.J. Bedard, Parkinsonism Relat. Disord. 10 (2004) 297–304.
[9] S. Przedborski, K. Tieu, C. Perier, M. Vila, J. Bioenerg. Biomembr. 36 (2004) 375–379.
[10] A. Schober, Cell Tissue Res. 318 (2004) 215–224.
[11] G.W. Ross, R.D. Abbott, H. Petrovitch, D.M. Morens, A. Grandinetti, K.H. Tung, C.M. Tanner, K.H. Masaki, P.L. Blanchette, J.D. Curb, J.S. Popper, L.R. White, JAMA 283 (2000) 2674–2679.
[12] A. Ascherio, S.M. Zhang, M.A. Hernan, I. Kawachi, G.A. Colditz, F.E. Speizer, W.C. Willett, Ann. Neurol. 50 (2001) 56–63.
[13] E.K. Tan, C. Tan, S.M. Fook-Chong, S.Y. Lum, A. Chai, H. Chung, H. Shen, Y. Zhao, M.L. Teoh, Y. Yih, R. Pavanni, V.R. Chandran, M.C. Wong, J. Neurol. Sci. 216 (2003) 163–167.
[14] J.F. Chen, K. Xu, J.P. Petzer, R. Staal, Y.H. Xu, M. Beilstein, P.K. Sonsalla, K. Castagnoli, N. Castagnoli Jr., M.A. Schwarzschild, J. Neurosci. 21 (2001) RC143.
[15] K. Xu, Y.H. Xu, J.F. Chen, M.A. Schwarzschild, Neurosci. Lett. 322 (2002) 13–16.
[16] M.S. Gevaerd, R.N. Takahashi, R. Silveira, C. Da Cunha, Brain Res. Bull. 55 (2001) 101–106.
[17] G.W. Ross, H. Petrovitch, Drugs Aging 18 (2001) 797–806.
[18] S. Shiozaki, S. Ichikawa, J. Nakamura, S. Kitamura, K. Yamada, Y. Kuwana, Psychopharmacology 147 (1999) 90–95.
[19] J.F. Chen, S. Steyn, R. Staal, J.P. Petzer, K. Xu, C.J. Van Der Schyf, K. Castagnoli, P.K. Sonsalla, N. Castagnoli Jr., M.A. Schwarzschild, J. Biol. Chem. 277 (2002) 36040–36044.
[20] M.A. Schwarzschild, K. Xu, E. Oztas, J.P. Petzer, K. Castagnoli, N. Castagnoli Jr., J.F. Chen, Neurology 61 (2003) S55–S61.
[21] M. Zdunek, J. Piosik, J. Kapuscinski, Biophys. Chem. 84 (2000) 77–85.
[22] J. Kapuscinski, B. Ardelt, J. Piosik, M. Zdunek, Z. Darzynkiewicz, Biochem. Pharmacol. 63 (2002) 625–634.

[23] J. Piosik, M. Zdunek, J. Kapuscinski, Biochem. Pharmacol. 63 (2002) 635–646.
[24] J. Piosik, K. Ulanowska, A. Gwizdek-Wisniewska, A. Czyz, J. Kapuscinski, G. Wegrzyn, Mutat. Res. 530 (2003) 47–57.
[25] A. Czyz, J. Jasiecki, A. Bogdan, H. Szpilewska, G. Wegrzyn, Appl. Environ. Microbiol. 66 (2000) 599–605.
[26] A. Czyz, H. Szpilewska, R. Dutkiewicz, W. Kowalska, A. Biniewska-Godlewska, G. Wegrzyn, Mutat. Res. 519 (2002) 67–74.
[27] G. Wegrzyn, A. Czyz, J. Appl. Microbiol. 95 (2003) 1175–1181.
[28] R. Belas, A. Mileham, D. Cohn, M. Hilman, M. Simon, M. Silverman, Science 218 (1982) 791–793.
[29] G. Klein, R. Walczak, E. Krasnowska, A. Blaszczak, B. Lipinska, Mol. Microbiol. 16 (1995) 801–811.
[30] A.S. Kalgutkar, S. Zhou, O.A. Fahmi, T.J. Taylor, Drug Metab. Dispos. 31 (2003) 596–605.
[31] J.R. Cashman, Toxicology 43 (1987) 173–182.
[32] A. Czyz, W. Kowalska, G. Wegrzyn, Bull. Environ. Contam. Toxicol. 70 (2003) 1065–1070.
[33] J. Kapuscinski, M. Kimmel, Biophys. Chem. 35 (1993) 46–53.
[34] R.W. Larsen, R. Jasuja, R.K. Hetzler, P.T. Muraoka, V.G. Andrada, D.M. Jameson, Biophys. J. 70 (1996) 443–452.
[35] D.B. Davies, D.A. Veselkov, L.N. Djimant, A.N. Veselkov, Eur. Biophys. J. 30 (2001) 354–366.
[36] A.N. Veselkov, M.P. Evstigneev, D.A. Veselkov, D.B. Davies, J. Chem. Phys. 115 (2001) 2252–2266.
[37] D.B. Davies, D.A. Veselkov, M.P. Evstigneev, A.N. Veselkov, J. Chem. Soc. Perkin Trans. 2 (2001) 61–67.
[38] S.C. Yang, S.P. Markey, K.S. Bankiewicz, W.T. London, G. Lunn, Lab. Anim. Sci. 38 (1988) 563–567.
[39] R.R. McCusker, B.A. Goldberger, E.J. Cone, J. Anal. Toxicol. 27 (2003) 520–522.
[40] M. Thiruchelvam, E.K. Richfield, B.M. Goodman, R.B. Baggs, D.A. Cory-Slechta, Neurotoxicology 23 (2002) 621–633.
[41] J.K. Andersen, Neurotoxicol. Res. 5 (2003) 307–313.
[42] B.K. Barlow, E.K. Richfield, D.A. Cory-Slechta, M. Thiruchelvam, Dev. Neurosci. 26 (2004) 11–23.
[43] J.A. Firestone, T. Smith-Weller, G. Franklin, P. Swanson, W.T. Longstreth Jr., H. Checkoway, Arch. Neurol. 62 (2005) 91–95.
[44] A. Storch, S. Ott, Y.I. Hwang, R. Ortmann, A. Hein, S. Frenzel, K. Matsubara, S. Ohta, H.U. Wolf, J. Schwarz, Biochem. Pharmacol. 63 (2002) 909–920.
[45] H. Elkon, E. Melamed, D. Offen, Mol. Neurosci. 24 (2004) 387–400.
[46] T.R. Guilarte, Is methamphetamine abuse a risk factor in parkinsonism? Neurotoxicology 22 (2001) 725–731.