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THE EFFECT OF CYCLOHEXIMIDE ON BUTYRYLCHOLINESTERASE ACTIVITY *IN VIVO*

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The paper describes the catalytic activity of butyrylcholinesterase (BuChE) measured in plasma, liver, white adipose tissue, heart, and brain of rats intraperitoneally administered a single non–lethal dose of cycloheximide (2.0 mg/kg body weight; CHM). The BuChE assay was performed on rats of both sexes either administered CHM or saline (controls), and killed 2, 3, 4, 5, 10 days later. A significant decrease of BuChE catalytic activity was observed in all tested tissues except plasma. In animals of both sexes, the lowest BuChE catalytic activity was found in the liver (2–6%), while it was higher in white adipose tissue, heart, and brain. However, the respective values remained significantly different from controls (33–67%, 49–62%, and 14–71% in males, and 24–82%, 72–86%, and 33–67% in females). Since there was no effect of CHM on BuChE catalytic activity in plasma, the data suggest that CHM inhibits the synthesis of BuChE rather than its active site.

KEY WORDS: *brain, heart, liver, plasma, protein synthesis, rat, white adipose tissue*

The toxicological importance of butyrylcholinesterase (BuChE: EC 3.1.1.8, acylcholine acylhydrolase, plasma cholinesterase) is evident in the fact that the diagnosis of poisoning by organophosphorous compounds (OPs) relies on clinical symptoms and laboratory tests for plasma BuChE and red cell acetylcholinesterase (AChE: EC 3.1.1.7) catalytic activity. Butyrylcholinesterase is synthesized in the liver and is released in blood (1), but some authors (2) propose that its synthesis is not limited to the liver alone. The enzyme has been found in the small intestine, smooth muscle, adipose tissue, heart, and white matter of the brain. It is not known whether the catalytic activity of BuChE in these tissues contributes to the enzyme activity in plasma. So far, BuChE has no known biological substrate in the mammals (3), but it hydrolyses a variety of esters of choline including butyrylcholine, butyrylthiocholine, propionylthiocholine (4, 5). Butyrylcholinesterase is clinically important inasmuch as it hydrolyses the short–acting muscle relaxant succinylcholine and the ester type of local anaesthetics (5, 6). Although the physiological function of BuChE is not known, it was suggested to be a precursor of AChE in the nervous system, and that it contributed to the integrity of the myelin sheath of the central axons (7). Some authors suggest that BuChE plays a role in lipid and lipoprotein metabolism (8–10).

Cycloheximide (CHM) (Figure 1) is an antibiotic substance obtained from streptomycin–producing strains of *Streptomyces griseus* (11). Cycloheximide had been used in the treatment of cryptococcal infections (*Cryptococcus neoformans*) in humans before the development of amphotericin B. Cycloheximide has antifungal properties and has commercially been used in the USA to treat various fungal infections of plants and to regulate plant growth (11, 12). Therefore, to workers in agriculture, CHM presents a serious risk of poisoning.

Cycloheximide is highly toxic (rat oral LD_{50} is 2.7 mg/kg, intraperitoneal LD_{50} is 3.7 mg/kg) and strongly inhibits protein synthesis in mammals and mammalian cell culture systems (13, 14). However, Devasagayam and co–workers (15) observed that CHM (2.0 mg/kg b.w.) induced the synthesis of certain microsomal proteins of the rough endoplasmatic reticulum in the rat liver within the first two hours. The reversal, that is, the significant inhibition of the synthesis occurred in the 24 hours that followed.

Generally, there are few data about the influence of CHM on BuChE activity. The results of Gupta and Dettbarn (16) have shown that CHM (0.5 mg/kg) administered to rats subcutaneously for four days did not alter AChE and BuChE catalytic activities in the brain and muscle. However, the total protein synthesis in the brain, muscle, liver and kidney significantly dropped.

The inhibition of protein synthesis by CHM and the toxicological and pharmacological importance of BuChE encouraged us to investigate how a non–lethal CHM dose can affect BuChE catalytic activity in the liver and other tissues. Since BuChE catalytic activity is a good parameter of exposure to various chemicals (organophosphorus and carbamate esters), the other aim of our study was to see whether it could be used as an indicator of CHM poisoning.

MATERIAL AND METHODS

Chemicals

Cycloheximide 3–(2R)–2–[(1S,3S,5S)–3,5–dime−thyl–2–oxocyclohexyl]–2–hydroxyethylglutarimide ob−tained from Sigma Chemical Co. (USA) was dissolved in saline (1.0 mg/ml) (Figure 1).

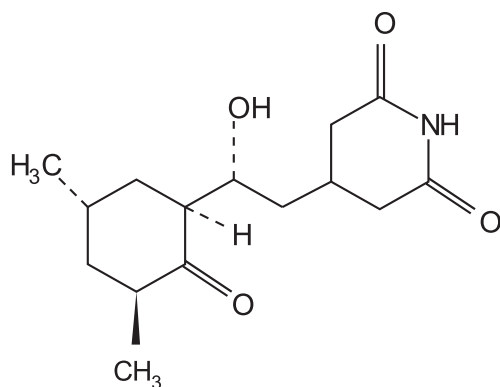**Figure 1** *Structural formula of cycloheximide*

Treatment of animals

Adult male and female Wistar rats (240–280 g body weight) were fed on a standard diet for laboratory rodents (Sljeme, Zagreb, Republic of Croatia). Animals had free access to water and were kept in macralone cages under controlled conditions (room temperature 21 °C, light and dark cycle exchanging every 12 hours). The rats (40 males and 40 females) were randomised in five control groups (n=3 each), and five experimental groups (n=5 each) receiving a single intraperitoneal (i.p.) dose of CHM (2.0 mg/kg body weight) on the first day of the experiment. The controls were given saline according to the same experimental design.

All animals were killed with coal gas 2, 3, 4, 5 and 10 days after CHM/saline administration. Blood samples were obtained directly from the heart, and the adipose tissue was isolated from the epididymal (males) or parametrial (females) fat depots. Heart, brain and liver tissues were rinsed with saline. Plasma and tissue samples were frozen immediately after sampling at –20 °C until further processing. The tissue samples were homogenised (200 mg tissue/ml saline), and centrifuged at 2800 x g for 15 min to obtain the supernatant.

BuChE analysis

The catalytic activity of this enzyme in plasma and heart, brain, liver, and white adipose tissues was determined by spectrophotometry (17), using butyrylthiocholine (0.9 mM) (Sigma ChemCo, St. Louis, USA) as the substrate. Since heart, brain, and liver tissues contain BuChE and AChE, the BuChE assay was carried out with and without the specific BuChE inhibitor ethopropazine hydrochloride in the final concentration of 1.7 μM (Sigma ChemCo, St. Louis, USA). The catalytic activity of BuChE was calculated indirectly as the difference between the two measurements. The activity of the enzyme is expressed as μmol of substrate hydrolysed/min/ml for plasma and as μmol of substrate hydrolysed/min/g for the tissues. Since controls of the same sex did not differ in the catalytic activity of BuChE in either plasma or the tissues, we took the mean BuChE catalytic activity of all male or female rats as control values. Relative changes in the enzyme activity in the treated animals are presented as the percentage of activity of the respective control group.

Statistical analysis

Data are shown as means and standard deviations (SD). Means, medians, and SD were calculated using a software package Statistics® for Windows Version 5.0 A (18). The statistical significance was determined by the parametric t–test and non–parametric Kolmogorov–Smirnov two–sample test, where appropriate. The differences discussed in this paper were considered significant at P<0.05 level of significance.

RESULTS AND DISCUSSION

The mean BuChE catalytic activity in plasma, liver and white adipose tissue was significantly (1.5–3 times) higher in female than in male control rats. This sex–related difference is in accordance with the results published elsewhere (19, 20). These results show a negative modulatory effect of testosterone and a positive modulatory effect of estrogens on serum BuChE synthesis in the rat liver. The mode of action of both hormones on the enzyme synthesis in hepatocytes is not direct, but includes hypothalamic–hypophyseal axis (21).

Tables 1 and 2 show the results of the BuChE assay in plasma, liver, heart, brain, and white adipose tissue of male and female rats obtained in our experiments. Although BuChE catalytic activity was significantly higher in female than in male controls in certain tissues, the percentage of BuChE catalytic activity observed in treated animals was consistent with control values for the same sex throughout the experiment.

Throughout the experiment, a single non–lethal dose of CHM (2.0 mg/kg body weight) significantly inhibited BuChE catalytic activity in the liver of both sexes. Since CHM inhibits protein synthesis (22) and BuChE is synthesised in the liver, the drop in the catalytic activity of BuChE may be attributed to the CHM inhibition of BuChE synthesis. The CHM inhibition of protein synthesis in the liver of rats given a lethal i.p. dose of CHM (10 mg/kg) was near complete after 3 hours and remained such for 9 hours (time to death was 14–20 hours after CHM) (23). As BuChE catalytic activity in our experiment did not recover for as long as 10 days after the treatment, we believe that the delay of the *de novo* synthesis of BuChE in rats is longer than reported elsewhere.

After the synthesis in the liver, BuChE is released into plasma. The biological half–life of BuChE in rat plasma is 9–11 days (24). After CHM treatment, BuChE catalytic activity in the plasma of rats of both sexes remained unchanged throughout the experiment. Although the synthesis of BuChE in the liver was completely inhibited by CHM, we believe that its relatively long half–life accounts for a maintained catalytic activity in plasma.

Table 1 *The catalytic activity of BuChE (Mean ± SD) and relative changes in the plasma and tissue of male rats killed 2, 3, 4, 5, and 10 days after a single cycloheximide doze (2.0 mg/kg b.w.; i.p.)*

Specimens	Units	Control animals N=15	Experimental animals time of sacrifice (days after treatment)				
			2 N=5	3 N=5	4 N=5	5 N=5	10 N=5
Plasma	μmol/ min/ml (%)	0.05±0.01 (100)	0.05±0.01 (100)	0.05±0.01 (100)	0.04±0.00 (80)	0.04±0.01 (80)	0.04±0.01 (80)
Liver	μmol/ min/g tissue (%)	0.36±0.01 (100)	0.01±0.00++ (3)	0.01±0.00++ (3)	0.01±0.00*** (3)	0.01±0.00*** (3)	0.02±0.00++ (6)
White adipose tissue		0.33±0.17 (100)	0.18±0.01+ (55)	0.22±0.07 (67)	0.19±0.02 (58)	0.15±0.01* (45)	0.11±0.01+ (33)
Heart		0.61±0.09 (100)	0.36±0.09** (59)	0.38±0.05* (62)	0.30±0.09** (49)	0.32±0.08** (52)	0.34±0.07** (56)
Brain		0.07±0.02 (100)	0.03±0.01*** (43)	0.05±0.02 (71)	0.03±0.00** (43)	0.01±0.00*** (14)	0.03±0.00** (43)

Legend: significantly different from control groups, *t–test P<0.05; **t–test P<0.01; ***t–test P<0.001; +Kolmogorov–Smirnov test P<0.01; ++Kolmogorov–Smirnov test P<0.001.

Table 2 *The catalytic activity of BuChE (Mean ± SD) and relative changes in the plasma and tissue of female rats killed 2, 3, 4, 5, and 10 days after a single cycloheximide doze (2.0 mg/kg b.w.; i.p.)*

Specimens	Units	Control animals N=15	Experimental animals time of sacrifice (days after treatment)				
			2 N=5	3 N=5	4 N=5	5 N=5	10 N=5
Plasma	μmol/ min/ml (%)	0.15±0.00 (100)	0.11±0.02 (73)	0.15±0.05 (100)	0.10±0.00 (67)	0.15±0.02 (100)	0.14±0.08 (93)
Liver	μmol/ min/g tissue (%)	0.50±0.05 (100)	0.01±0.00[+++] (2)	0.03±0.00[+++] (6)	0.03±0.00[+++] (6)	0.03±0.00[++] (6)	0.02±0.01[***] (4)
White adipose tissue		0.45±0.06 (100)	0.23±0.04[+] (51)	0.26±0.06 (58)	0.37±0.07 (82)	0.11±0.03[**] (24)	0.17±0.05[**] (38)
Heart		0.64±0.10 (100)	0.69±0.04 (108)	0.49±0.03[+] (77)	0.55±0.09 (86)	0.46±0.01 (72)	0.47±0.05 (73)
Brain		0.06±0.02 (100)	0.03±0.00[*] (50)	0.03±0.01[**] (50)	0.02±0.00[***] (33)	0.03±0.00[*] (50)	0.04±0.00 (67)

Legend: significantly different from control groups, [*]t–test $P<0.05$; [**]t–test $p < 0.01$; [***]t–test $P<0.001$; [+]Kolmogorov–Smirnov test $P<0.05$; [++] Kolmogorov–Smirnov test $P<0.01$; [+++]Kolmogorov–Smirnov test $P<0.001$.

A significant drop in BuChE activity was also observed in white adipose tissue, heart and brain (40–65% of control values). However, relative BuChE activity in these tissues was higher than in the liver. Cycloheximide is an imide whose metabolism is not fully understood. In the endoplasmatic reticulum of hepatocytes in humans and rats, imides are hydroxylated to more polar compounds with lower toxicity (25, 26). Literature has little data on BuChE catalytic activity in the adipose tissue and heart in CHM–treated animals. Gupta and Dettbarn (16) reported that subcutaneous administration of CHM (0.5 mg/kg for 4 days) did not significantly alter BuChE catalytic activity in either brain or other investigated tissues. It is likely that these authors administered subcutaneous no–effect doses of CHM for BuChE activity. We therefore believe that the difference between our and their results is due to a difference in doses. However, the route (intraperitoneal vs. subcutaneous administration), and the number of doses (multiple vs. single) can not be ruled out as reasons. As in the liver, the absence of BuChE recovery in the adipose tissue, heart and brain showed a long–lasting enzyme inhibition caused by CHM at the end of the experiment.

In our study, CHM was administered intraperito–neally and was primarily absorbed through the portal circulation. In other words, it had to pass the liver before it reached other organs. The phenomenon of reduced pharmacological or toxicological effect of various xenobiotics in other organs due to their detoxification in the liver, known as the first–pass effect, is the plausible explanation for higher BuChE catalytic activity in white adipose tissue, heart and brain than in liver.

This is the first report on the effects of CHM on BuChE activity in animal plasma, liver, white adipose tissue and heart. Our findings on BuChE catalytic activity corroborate authors who suggest that CHM inhibits protein synthesis. This conclusion is seconded by the differences in BuChE catalytic activity between plasma and liver or other tissues, which indicate that it is the synthesis, rather than the enzymes catalytic activity which is being inhibited by CHM. The activity of BuChE in plasma is therefore not a suitable marker of CHM poisoning.

Acknowledgement

REFERENCES

1. Whittaker A. Plasma cholinesterase variants and the anaesthesist. Anaesthesia 1980;35:174–97.
2. Orešković K, Kunec–Vajić E. Pseudocholinesterase in aloxan–diabetic rats. Res Commun Chem Pathol Pharmacol 1992;78:117–20.
3. Kutty KM, Annapurna V, Prabhakaran V. Pseudocholinesterase: A protein with functions unrelated to its name. Biochem Soc Trans 1989;17: 555–6.
4. Brown SS, Kalow W, Pilz W, Whitaker M, Woronick CL. The plasma cholinesterases: a new perspectives. Adv Clin Chem 1981;22:121.
5. Kalow W. Hydrolysis of local anesthetics by human serum cholinesterase. J Pharmacol Exp Ther 1952;104:122–34.
6. Kunec–Vajić E, Bradamante V, Uroić B. The effect of local anesthetics and phenothiazine derivatives on cholinesterase, investigated by a chemiluminescence method. Acta Pharm Jugosl 1985; 35:133–36.
7. Kutty KM, Payne RH. Serum pseudocholinesterase and very–low–density lipoprotein metabolism. J Clin Lab Anal 1994;8:247–50.
8. Kutty KM. Biological function of cholinesterase. Clin Biochem 1980;13:239–43.
9. Kutty KM, Redheendran R, Murphy D. Serum cholinesterase: Function in lipoprotein metabolism. Experientia 1977;33:420–1.
10. Krnić Ž, Bradamante V. Effects of Oxprenolol treatment on pseudocholinesterase and lipids in rats. Arzneim Forsch Drug Res 1997;47:910–3.
11. Budavari S. The Merck index. 11th edition. New Jersey (NJ): Rahvay; 1989.
12. Gosselin E, Smith RP, Hodge HC. Clinical Toxicology of Commercial Products. 5th edition. Baltimore (MD): Williams and Wilkins; 1984.
13. Young CW, Dowlin MD, Jr. Antipyretic effect of cycloheximide, an inhibitor of protein synthesis in patients with Hodgkin's disease or other malignant neoplasms. Cancer Res 1975;35:1218–24.
14. Young CW, Robinson PF, Sacktor B. Inhibition of the synthesis of protein in intact animals by acetoxycycloheximide and a metabolic derangement concomitant with this blockade. Biochem Pharmacol 1963;12:855–65.
15. Devasagayam TPA, Pushpindran Ck, Eapen J. Induction of rat liver microsomal enzymes by cycloheximide. Biochem Pharmacol 1979;28:1731–4.
16. Gupta RC, Dettbarn W.D. Interaction of cycloheximide and diisopropylphosphorofluoridate (DFP) during subchronic administration in rat. Toxicol Appl Pharmacol 1987;90:52–9.
17. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.
18. Statsoft Inc. Statistica for Windows [computer program manual]. Tulsa (OK): StatSoft Inc.; 1995
19. Illsley NP, Lamartiniere AC. Endocrine regulation of rat serum cholinesterase activity. Endocrinology 1981;108:1737–43.
20. Schmidt E, Schmidt FW. Sex differences of plasma cholinesterase in the rat. Enzyme 1978;23:52–5.
21. Edwards JA, Brimijoin S. Effects of hypophysectomy on acetylcholinesterase and butyrylcholinesterase in the rat. Biochem Pharmacol 1983;32:1183–9.
22. Helinek TG, Devlin TM, Ch'ih JJ. Initial inhibition and recovery of protein synthesis in cycloheximide–treated hepatocytes Biochem Pharmacol 1982;31:1219–25.
23. Thompson WL, Wannemacher RW Jr. In vivo effects of T–2 mycotoxin on synthesis of proteins and DNA in rat tissues. Toxicol Appl Pharmacol 1990;105:483–91.
24. Schneider LS, Giacobini E. Metrifonate: a cholinesterase inhibitor for Alzheimer's disease therapy. CNS Drug Reviews 1999;5:13–26.
25. Testa B, Jenner P. Drug metabolism: chemical and biochemical aspects. In: Swarbrick J, editor. Drugs and the pharmaceutical science. 4th edition. New York (NY): Marcel Dekker Inc.; 1976. pp. 53–166.
26. Mutschler E, Derendorf H. Drug action: basic principles and therapeutic aspects. Stuttgart (GE): Medpharm Scientific Publishers; 1995.

Sažetak

DJELOVANJE CIKLOHEKSIMIDA NA AKTIVNOST BUTIRILKOLINESTERAZE *IN VIVO*

U ovom je istraživanju mjerena katalitička aktivnost enzima butirilkolinesteraze (BuChE) u plazmi, jetri, bijelom masnom tkivu, srcu i mozgu štakora tretiranih jednokratnom intraperitonealnom dozom cikloheksimida (2,0 mg/kg tjelesne težine, CHM). Katalitička aktivnost enzima izmjerena je u navedenim tkivima životinja obaju spolova koje su žrtvovane 2, 3, 4, 5 ili 10 dana nakon primjene CHM–a ili fiziološke otopine (kontrolna skupina). Značajno smanjenje katalitičke aktivnosti enzima BuChE izmjereno je u svim tkivima tretiranih životinja osim u plazmi gdje njegova aktivnost u životinja obaju spolova tijekom cjelokupnog istraživanja nije značajno odstupala od kontrolnih vrijednosti. Preostala katalitička aktivnost BuChE u jetrenome tkivu tretiranih životinja obaju spolova bila je 2–6%, dok je u masnom tkivu, srcu i mozgu aktivnost ovog enzima bila viša negoli u jetri, no statistički značajno različita od aktivnosti u istim tkivima kontrolnih životinja. Katalitička aktivnost BuChE u bijelome masnom tkivu, srcu i mozgu tretiranih mužjaka bila je 33–67, 49–62 i 14–71% u odnosu na aktivnost enzima u tkivima kontrolnih mužjaka, dok je u istim tkivima ženki taj raspon bio 24–82, 72–86 i 33–67%. Butirilkolinesteraza je enzim koji se većim dijelom sintetizira u jetri te izlučuje u cirkulaciju gdje mu je vrijeme polovičnog raspada 8–12 dana. Budući da CHM nije imao učinka na katalitičku aktivnost BuChE u plazmi štakora, a aktivnost enzima u jetri i drugim tkivima u kojima se vjerojatno sintetizira bila je značajno smanjena, može se zaključiti da CHM inhibira sintezu enzima, a ne njegovu katalitičku aktivnost. Stoga aktivnost BuChE u plazmi ne može biti pokazatelj otrovanja cikloheksimidom u ljudi.

KLJUČNE RIJEČI: *bijelo masno tkivo, jetra, mozak, plazma, sinteza proteina, srce, štakor*

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