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Correlation between serum butyrylcholinesterase activity and serum lipid concentrations in rats treated with different antagonists of the adrenergic system

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Abbreviations
BuChE = butyrylcholinesterase
HDL = High-density lipoprotein
LDL = Low-density lipoprotein
VLDL = Very-low-density lipoprotein
TC = total cholesterol concentration
TG = triglyceride concentration
HDL-C = HDL cholesterol concentration

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Abstract

Background and Purpose: *Based on the facts that the blockade of adrenergic receptors can alter lipid profile in the serum and that it has been suggested that butyrylcholinesterase (BuChE) is involved in lipid metabolism, different adrenergic blocking agents were administered to rats to modify lipid concentrations in serum. The activity of BuChE was examined under such conditions and correlations with serum lipids were investigated. The purpose of this study was to evaluate the effects of different adrenergic antagonists on BuChE activity and to investigate the correlation between BuChE activity and serum lipids.*

Materials and Methods: *Six groups of male Fischer 344 rats (9 animals/group) were treated orally with adrenergic antagonists (mixed in commercial diet) during 6 weeks: oxprenolol, atenolol, doxazosin, oxprenolol and doxazosin, atenolol and doxazosin, and guanethidine. A control group (9 rats) received only commercial diet. BuChE activity in serum was determined with kinetic color test using butyrylthiocholine as a substrate. Concentrations of serum lipids (total cholesterol, triglycerides and HDL cholesterol) were determined by enzymatic colorimetric tests. Data were analyzed by Kruskal-Wallis test and Spearman's correlation coefficient.*

Results: *The results revealed that oxprenolol and doxazosin (given alone or in combination with atenolol or oxprenolol) increased (>30 %) BuChE activity. BuChE activity correlated with different serum lipids, and correlation depended on the type of adrenergic blockade.*

Conclusion: *Although the examined adrenergic antagonists did not influence serum lipid concentrations, the increase of BuChE activity and correlation with serum lipid concentrations suggested that the increase of this enzyme's activity might be the first sign of altered lipid metabolism.*

INTRODUCTION

Butyrylcholinesterase (BuChE, pseudocholinesterase, EC 3.1.1.8) is a serum esterase which is synthesized primarily in the liver *(1)* and released into plasma immediately following its synthesis. This enzyme is also found in the small intestine, smooth muscle, adipose tissue, brain and other tissues, but it is not known whether this enzyme originates only from blood, or whether it can be synthesized in those tissues as well. The true physiological function of BuChE has not yet been identified. It was suggested that it is a precursor of acetylcholinesterase

(AChE, EC 3.1.1.7) in the nervous system, with an important role in the regulation of slow impulse conduction in the nervous system, and that it is included in the hydrolysis of ingested esters from plant sources *(2, 3)*. On the other hand, the clinical importance of BuChE is well known. It hydrolyzes muscle relaxant succinylcholine and local anesthetics like procaine and tetracaine hydrochloride *(4, 5)*. When plasma BuChE activity is low, as the result of inadequate hepatic synthesis or in the case of abnormal genetic variants, the metabolism of succinylcholine is reduced resulting in the increase in the duration of muscular relaxation and prolonged respiratory paralysis. Because BuChE activity in plasma can reflect the rate of its formation in hepatocytes, quantitative determination of the catalytic activity of BuChE in serum and plasma may be used as a biomarker to identify liver disorders. A decrease in BuChE activity in plasma is an indicator of pesticide poisoning.

Results of some investigators have shown that BuChE is probably involved in lipid metabolism. Clitherow et al suggested that BuChE might hydrolyze butyrylcholine possibly formed during fatty acid metabolism *(6)*. Ballantyne *et al.* showed that BuChE occurred in the sebaceus gland and adipose tissue and suggested that BuChE took part in lipid metabolism *(7, 8)*. An increased serum BuChE activity is usually observed in conditions associated with altered lipid metabolism such as hyperlipoproteinemia, obesity and diabetes *(3, 9–17)*. Thus, increased activity of BuChE was found when triglyceride (TG), very low density lipoprotein (VLDL) or low density lipoprotein (LDL) concentrations were increased in animal models of diabetes and obesity *(9–11)*. Increased activity can be found in patients with diabetes *(13, 14),* obesity and hyperlipoproteinemia *(15, 16, 17)* as well.

It is well known that α and β adrenergic receptor antagonists modify the metabolism of carbohydrates and lipids, when they are used in patients *(18–28)* for treatment of hypertension. The reported data about the effect of adrenergic receptor antagonists on lipid metabolism are conflicting and different. While selective β_1 and nonselective $\beta_{1,2}$ adrenergic receptor antagonists mostly increase TG and/or LDL cholesterol level and reduce HDL cholesterol *(20–25, 28),* those that act as α_1 adrenergic receptor antagonists can increase HDL cholesterol (HDL-C) and decrease total cholesterol (TC) or TG concentrations *(18–21, 25–28)*. The sympathetic system is known to have several of important effects on metabolic processes in adipose tissue. So it has been suggested that catecholamines are of major importance for the regulation of lipolysis in adipose tissue. In human fat cells, both β_1 and β_2 adrenergic receptors are known to stimulate cAMP production and lipolysis *in vitro* and *in vivo (29, 30)*. β_3 adrenergic receptors are highly expressed in rodent white and brown adipose tissue and are relatively specific for this tissue. Selective agonists of β_3 adrenergic receptors elicit a marked lipolytic and thermogenic response in rodents *(29, 30)*. According to literature data, the level of expression and the contribution of the β_3 receptor to catecholamine-induced lipolysis in human subcutaneous adipocytes seem to be limited *(31)*. Antilipolytic effect of catecholamines is exerted through the stimulation of α_2 adrenergic receptors on fat cells *(29, 32)*. It was suggested that catecholamines have a higher affinity for α_2 than for β adrenergic receptor, and that they are responsible for α adrenergic pathways in the control of lipolysis in humans *(33)*.

Propranolol, a nonselective β adrenergic receptor antagonist, was shown to inhibit BuChE activity *in vitro (34, 35),* but also *in vivo* in rats (brain tissue) *(35)*. In contrast, the results of our investigations showed that the nonselective β adrenergic receptor antagonist oxprenolol significantly increased BuChE activity in rats of both sexes when given for a long period of 6–12 weeks *(36, 37)*. As serum TG or TC concentrations were altered in these experiments, it was concluded that the increase of enzyme activity was due to the altered lipid metabolism caused by oxprenolol rather than its direct effect on the enzyme.

Different adrenergic receptor antagonists are commonly used for treatment of cardiovascular diseases, so it was of interest to assess whether other adrenergic antagonists also influenced serum BuChE activity. It was also of interest to examine if there was any correlation between serum BuChE activity and serum lipid concentrations (TG, TC and HDL-C). For this purpose, adrenergic antagonists with different site of action were used in the experiment: non-selective β_1 and β_2 adrenergic receptor antagonist oxprenolol, selective β_1 adrenergic receptor antagonist atenolol, α_1 adrenergic receptor antagonist doxazosin and adrenergic neuron-blocking agent guanethidine.

MATERIAL AND METHODS

Test substances

Test substances oxprenolol hydrochloride (CAS 6452--71-7), atenolol (CAS 29122-68-7), and doxazosin mesylate (CAS 77883-43-3) were donated by PLIVA d.d. (Zagreb, Croatia). Guanethidine monosulfate (CAS 645-43-2) was obtained from Sigma.

Animals

Male Fischer 344 rats (PLIVA Research Institute), 3 months old, with average weight of 270 g were used in the experiment. The animals were housed (3 rats/cage) in makrolon cages (dimension 425x266x180 mm). The cages were located in rooms under controlled conditions (12h light: 12h dark, temperature 22 °C ±3 and relative humidity 55% ± 10). Before the treatment started, the animals were randomized according to their body weights. Housing, handling and treatment of animals were conducted on the basis of the current guide and directive for laboratory animals *(38, 39)*.

Study design and dosage

The animals were divided in seven groups (9 rats/group). Six groups were treated with tested substances for 6 weeks as follows: 1). group with oxprenolol; 2). with

atenolol; 3). with doxazosin; 4).with oxprenolol and doxazosin; 5). with atenolol and doxazosin; and 6). group with guanethidine. The substances were mixed in commercial diet for laboratory mice and rats (manufactured by PLIVA Veterina i agrar) and offered to animals *ad libitum*. The calculated average doses at the end of the treatment period were 9.6, 5.5, 1.9, and 2 mg/kg/day for oxprenolol, atenolol, doxazosin and guanethidine, respectively. The doses of antagonists were at least 2 times higher than maximum recommended human doses (mg/kg body weight). The seventh group of animals belonged to the control group and the rats were fed *ad libitum* only with commercial diet for laboratory animals (manufactured by PLIVA Veterina i agrar). The animals from all groups had free access to tap water (bottles). At the end of the treatment period the animals were anesthetized with overdose of barbiturate thiopental and blood samples were obtained from carotid artery. The serum was stored at –20 °C until analyzed.

Measurement of BuChE activity in serum

BuChE activity (U/L) in serum was determined on an automatic biochemical analyzer using a commercial kit with butyrylthiocholine as a substrate (OLYMPUS System Reagent Cholinesterase).

Measurement of serum lipid concentrations

Concentrations of TG and TC in serum were determined on automatic biochemical analyzer using enzymatic colorimetric tests and commercial kits OLYMPUS System Reagent 500 for triglycerides and cholesterol. The concentration of HDL-C was determined by enzymatic colorometric tests in the supernatant (HERBOS DIJAGNOSTIKA) after the precipitation of VLDL and LDL with polyethylene glycol (QUANTOLIP HDL, IMMUNO AG). The concentrations of serum lipids were expressed in mmol/L.

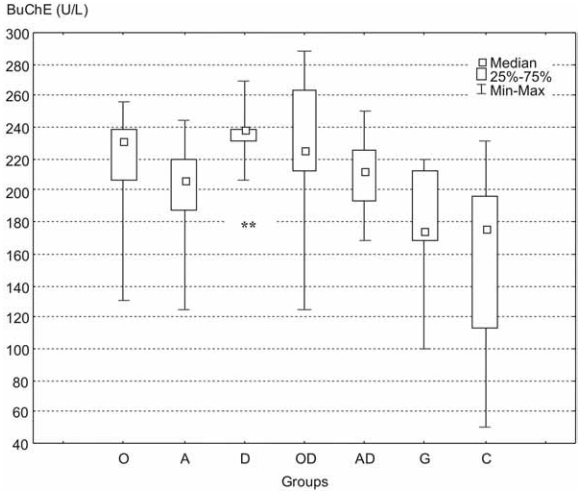

Figure 1. *Box and whisker plot of the BuChE activity (U/L) in male Fischer 344 rat serum in 7 groups after 6 weeks treatment: 9.6 mg oxprenolol/kg/day (O), 5.5 mg atenolol/kg/day (A), 1.9 mg doxazosin/kg/day (D), 9.6 mg oxprenolol and 1.9 mg doxazosin/kg/day (OD), 5.5 mg atenolol and 1.9 mg doxazosin/kg/day (AD), 2 mg guanethidine/kg/day (G) and control group (C). Statistical significance (**P<0.01) showed differences between the control group and the treated group. The small box is median, bigger box is the upper and lower quartiles (25 %-75 %), and the vertical bars are the full range of values in the data minimum and maximum (Kruskal-Wallis test).*

Statistical analysis

Kruskal-Wallis test was used to compare BuChE activity between groups. Data are shown as median, quartiles (25% and 75%), minimum and maximum.

Correlations between BuChE activity and the concentrations of TG, TC or HDL-C were derived using Spearman's correlation.

Post-hoc test was used for group comparison. The results were considered significant with $p<0.05$.

The percentage (%) of change in enzyme activity or lipid concentrations *vs* control group was based on the difference between calculated mean values.

RESULTS

Effects on serum BuChE activity

Box and whisker plot of the BuChE activities in rat serum in 7 groups after 6 weeks of treatment are presented in Figure 1. The administration of oxprenolol at the dose of 9.6 mg/kg/day induced the increase in serum BuChE activity of 39.8 % compared to the control group. Atenolol administered at the dose of 5.5 mg/kg/day induced the increase of 27.9% compared to the control group. A statistically significant increase in BuChE activity ($p<0.01$) was observed when doxazosin was given at the dose of 1.9 mg/kg/day. Doxazosin increased the enzyme activity by 51.6% compared to the control group. Concurrent administration of oxprenolol and doxazosin induced the increase in BuChE activity of 44.4% compared to the control group. When atenolol and doxazosin were given concurrently, the increase in BuChE activity of 34.2% (*vs* control) was observed. The measured BuChE activity in guanethidine-treated group (2 mg/kg/day) was similar to those of the control group. The increase was about 10.2%.

Effects on serum lipid concentration

No significant alteration in any of the examined lipid concentrations was observed in the experiment. The highest obtained change (either increase or decrease) was a decrease in TG by 19 % *vs* control in the doxazosin-treated group.

Correlation between BuChE activity and serum lipids

The correlations of serum BuChE activity with serum lipid concentrations (TC, TG or HDL-C) in rats are presented in Table 1. Positive correlations between serum BuChE activity and TC ($p<0.01$) or HDL-C ($p<0.05$) was found in the oxprenolol treated group. In the atenolol

TABLE 1

Correlation of serum BuChE activity with serum lipid concentrations (total cholesterol, triglycerides or HDL cholesterol) in male Fischer 344 rats (number of samples (N) = 8–9/group) after 6 weeks of treatment with different adrenergic antagonists (oxprenolol, atenolol, doxazosin, oxprenolol and doxazosin, atenolol and doxazosin, or guanethidine) and in the control group. Spearman's correlation coefficient (ρ) was used to identify the relation between BuChE and the serum lipids and significance probabilities were calculated (*P<0.05; **P<0.01). Calculations were performed for each group separately and for all the groups together (irrespective of treatment).

Group (N)			Total cholesterol	Triglycerides	HDL cholesterol
Oxprenolol	BuChE	ρ	0.842(**)	–0.184	**0.703(*)**
(N=9)		P	0.004	0.635	0.035
Atenolol		ρ	0.428	**0.669(*)**	0.218
(N=9)		P	0.251	0.049	0.574
Doxazosin		ρ	0.452	–0.144	0.358
(N=9)		P	0.222	0.711	0.344
Oxprenolol & Doxazosin		ρ	0.150	**–0.669(*)**	0.639
(N=9)		P	0.700	0.049	0.064
Atenolol & Doxazosin		ρ	0.517	–0.395	0.346
(N=9)		P	0.154	0.293	0.362
Guanethidine		ρ	–0.112	0.072	0.228
(N=9)		P	0.774	0.854	0.555
Control group		ρ	0.506	0.429	0.467
(N=8)		P	0.201	0.289	0.243
All group together		ρ	**0.377(**)**	**–0.288(*)**	**0.357(**)**
(N=62)		P	0.002	0.023	0.004

treated group, positive correlation was found ($p<0.05$) between BuChE activity and TG concentration. No statistically significant correlation was observed between BuChE activity and any of the examined lipid concentrations in the doxazosin treated group. Concurrent administration of oxprenolol and doxazosin revealed negative correlation ($p<0.05$) between BuChE activity and TG concentration. When atenolol and doxazosin were given concurrently, no statistically significant correlation was observed between BuChE activity and examined lipids. Guanethidine treatment did not induce a statistically significant correlation between BuChE activity and the serum lipids examined. When all groups were evaluated together irrespective of treatment, positive correlations were obtained between BuChE activity and TC or HDL-C ($p<0.01$), but negative correlation was obtained between enzyme activity and TG ($p<0.05$).

DISCUSSION

The present results are in agreement with our earlier results obtained in rats of both sexes when we found that chronic oxprenolol treatment causes an increase in BuChE activity *(36, 37)* (Figure 1). Although the increase in BuChE activity was not significant, as it was in our earlier experiments, the increase was higher than 35% (39.8% *vs* control). We suggest that the increase was not significant because of the lower dose of oxprenolol used in the current study. Actually, the calculated dose levels which we used in our earlier experiments were higher (15 and 30 mg/kg/day) in comparison with the dose level in the current experiment (9.6 mg/kg/day) (see Material and Methods). Despite this difference in the significance of BuChE activity obtained in our present and earlier results, we considered that oxprenolol had a potential to increase the activity of BuChE. Although chronic oxprenolol treatment did not alter significantly any of the serum lipid concentrations, a positive correlation between BuChE activity and serum TC and HDL-C was obtained (Table 1). The positive influence of oxprenolol on TC and HDL-C concentrations in present results is congruent with the results of our earlier experiments *(36, 40)* when we found significantly higher concentrations of TC *(36)* and HDL-C *(40)* after chronic oxprenolol treatment in comparison with the control group. On the other hand, our experiments with oxprenolol and glibenclamide showed a significant decrease in HDL-C after oxprenolol treatment *(37)*. We suppose that the influence of oxprenolol on TC and HDL can vary and that it probably depends on some factors, such as specific cholesterol transport in the rat which is qualitatively various from humans *(41),* or dose level of oxprenolol used.

Our present results showed that oxprenolol did not alter serum TG concentration, which is opposite to our earlier results *(37)*. In our opinion, these opposite results are probably due to two factors. The first and most im-

portant factor is the lower dose level of oxprenolol and shorter treatment in comparison to a higher dose level and longer treatment (12 weeks) in our earlier experiments. The second factor may be a higher sensitivity of α_2-antilipolytic adrenoreceptors in adipose tissue and α_1 adrenergic receptor in rat hepatocytes *(42)* for catecholamine, which became visible in the present experiment due to unidentified reason. It is known that the effect of adrenergic receptor antagonists on blood lipid level diffeers. Our results also showed that the influence of oxprenolol on serum lipids is dissimilar. Although either TC or TG was increased, the activity of BuChE was always increased. Because of these different observations in serum lipid concentrations, further studies in this area are indicated.

Our results showed that atenolol did not cause a significant increase in BuChE activity. Although the increase of enzyme activity was below 30%, the coefficient of correlation was positive with serum TG, suggesting that the higher dose of atenolol or its longer administration would probably increase the TG concentration together with the increase in BuChE activity. This is congruent with the results of Gaafar *et al.* who showed that atenolol at a dose of 9 mg/kg/day caused an increase in serum TG in healthy male rats after 30 days of treatment *(43)*. At this moment the full explanation of the mechanism of the TG modifying effects of atenolol remains unavailable since different factors can be responsible.

In humans, doxazosin as an α_1 adrenergic receptor antagonist usually decreases TG or TC and increases HDL-C *(21, 44)*. We would expect a decrease in BuChE activity after doxazosin treatment since positive correlation between BuChE and TG, or BuChE and TC, was found *(13, 14, 45)*. Surprisingly, 6 weeks of treatment with doxazosin increased BuChE activity, and this increase was statistically significant. Although it did not significantly change the serum lipid profile, doxazosin caused a decrease in serum TG by 19% *vs* control, and that was the highest obtained change (either increase or decrease) in the lipid concentration observed in this experiment. Recent data in experiments by α- and β-adrenoreceptor agonists in isolated rat hepatocytes also showed that β adrenergic receptors are involved in VLDL secretion, i.e. β adrenergic receptor agonist isoproterenol caused a significant inhibition of triglyceride secretion *(42)*. Their results were very useful in explaining the results of our experiments. In our experiments, we suggest that the decrease in TG concentration after doxazosin-treatment is a consequence of noradrenaline agonistic action on those adrenoreceptors which are responsible for antilipolytic effect of catecholamines (i.e β adrenergic receptors in hepatocytes and α_2 adrenergic receptors in adipose tissue). We do not know the reason why BuChE activity and TG concentration relation was not proportional.

As expected, guanethidine, which is an adrenergic neuron-blocking agent, did not have any influence on BuChE activity or serum lipid concentrations, and we did not find any significant correlation between BuChE activity and lipids. All the results were similar to those of the control group. Guanethidine is know to cause »pharmacological sympathectomy« and disappearance of noradrenaline in blood. We suggested that the low level of adrenaline that is secreted from adrenal medulla is sufficient for its permissive metabolic agonistic action on all free adrenergic receptors. Due to this reason the values of enzyme activity and serum lipids obtained in rats on guanethidine were similar to those obtained in the control group. Thus »pharmacological sympathectomy« is a possible explaination why the results after guanethidine treatment were similar to those obtained in the control group, but this is only a hypothesis and it should be further investigated.

A significant negative correlation between BuChE and TG was observed after treatment with doxazosin and oxprenolol combination, when $\beta_{1,2}$ and α_1 adrenergic receptors were blocked, but not in the case of β_1 and α_1 adrenergic receptor blockade (atenolol and doxazosin) (Table 1). The increase in BuChE activity was above 30% after both treatments, i.e. an increase in enzyme activity by 44.4% was observed after concurrent administration of oxprenolol and doxazosin and by 34.2% after administration of atenolol and doxazosin (Figure 1). According to these results, concurrent uses of doxazosin with oxprenolol or atenolol have no additional effect on serum BuChE and serum lipid concentration.

It is known that different adrenergic receptor antagonists alter serum lipid concentrations differently *(21)*. Our present results showed that they also had a different effect on BuChE activity. Although adrenergic receptors antagonists which we used in our experiments did not significantly alter serum lipid concentration, the increase in BuChE activity and demonstrable correlation with serum lipids suggested that the increase in enzyme activity might be the first sign of altered lipid metabolism. The results of the coefficient of correlation obtained from all groups together (Table 1), irrespective of the treatment, suggested that there was a positive correlation between the BuChE activity and TC and HDL-C in rat serum. Contrary to that, there was a negative correlation between BuChE activity and TG concentration.

CONCLUSION

Our results obtained in rats showed that some adrenergic receptor antagonists (doxazosin and oxprenolol) which we used in our experiments can increase serum BuChE activity. Although oxprenolol, atenolol and doxazosin, given alone or in combination, did not significantly influence serum lipid concentration, the increase in BuChE activity and the obtained correlation with certain serum lipids suggest that the increase in enzyme activity might be the first sign of altered lipid metabolism. We suppose that all these effects of adrenergic receptor antagonists, i.e. BuChE activity, serum lipid concentration and correlation between BuChE and lipids, depend on the type of adrenergic receptor and its antagonist. Our results also suggest that the measurement of BuChE activity during treatment with adrenergic

receptor antagonists is of clinical significance. A decrease or increase in BuChE activity can alter the metabolism of other drugs and, as a consequence, change the drug safety and efficacy (mainly when activity is decreased). Iatrogenic modification of BuChE can also influence diagnosis of certain conditions during which the enzyme activity is either decreased or increased.

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