Liver and serum butyrylcholinesterase activity in scorbutic guinea pigs

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The present study reports the effects of an ascorbic acid-deficient diet on liver and plasma butyrylcholinesterase activity. The activity of this enzyme decreased significantly in the plasma and liver subcellular fractions. The levels of plasma corticosterone were also significantly elevated in scurvy. The results are discussed in light of these observations.

Keywords: acetylcholinesterase; butyrylcholinesterase; ascorbic acid; guinea pig

Introduction

Cholinesterases are enzymes that hydrolyse esters of choline to produce choline and free acid. Two types of cholinesterases have been recognized in the mammalian systems. Acetylcholinesterase (acetyl-choline acetylhydrolase, EC 3.1.1.7) is responsible for the destruction of acetylcholine at the neuromuscular junction and is localized in the nervous system and the red blood cells. Butyrylcholinesterase (acetyl-choline acylhydrolase, EC 3.1.1.8) on the other hand is localized in various tissues, viz heart, liver, muscle, and intestine. Both types of enzyme catalyze the hydrolysis of acetylcholine but only the acetylcholinesterase acts on acetyl-β-methylcholine. In contrast, only butyrylcholinesterase acts on benzoylcholine and succinylcholine.

The activities of these enzymes have been reported to be altered in various nutritional disorders such as diabetes,[1] obesity and hyperlipemia,[2,3] avitaminosis,[4] and various other dietary diseases. The present study was undertaken to investigate the effects of avitaminosis C on liver and plasma butyrylcholinesterase activity in guinea pigs.

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Materials and methods

Studies on guinea pigs

In-bred strains of guinea pigs *(Cavia cobaya)* were used in these studies. The animals were divided into two groups, controls and scorbutics. The animals were approximately the same weights (300gm). Only male guinea pigs were used in these studies as the females have been shown to take longer to become deficient.[5] Vitamin C deficiency was induced by feeding the animals Lunde's Scorbutic Diet, *(Table 1)* as used by Ginter et al.[6] The diet was completely devoid of ascorbic acid and the control groups were administered 10 mg per day by stomach tube. The animals were fed ad libitum. Weights of the animals were measured daily to ensure that they were consuming the diet, and those that showed any weight loss were discarded. The diet was fed for 21–24 days, when scurvy developed. The animals were sacrificed on days 22–23, a period when the scorbutic group showed no weight loss or gain. The animals were sacrificed between 8:00 a.m. and 9:00 a.m.

Methods

The animals were sacrificed by cervical dislocation and the blood was collected by cutting the jugular veins in heparin. The liver tissue was removed and placed in ice-cold 0.25 M sucrose solution. The liver was washed in ice-cold 0.25 M sucrose and homogenized. The subcellular fractions were prepared by centrifugation. All procedures were carried out at 4° C.

Ascorbic acid in the plasma and liver fractions was determined by the method of Roe and Kuether[7] using 2,4-dinitro-phenylhydrazine. The butyrylcholinesterase was assayed by the procedure adopted by Ellman et al.[8] using butyrylthiocholine as a substrate. Corticosterone levels were

Table 1 Diets

Lunde's Scorbutic Diet	
Wheat bran	4,000 g
Oat flakes	10,000 g
Butter	2,000 g
Cod liver oil	25 mL
NaCl	200 g
Salt mixtures	100 g
Vitamin mixture	2,900 mg
Dried milk powder	3,000 g
Salt Mixture (Hegsted's Salt Mixture)	
$CaCO_3$	30.00 g
K_2HPO_4	32.25 g
NaCl	16.75 g
$CaHPO_4$	7.50 g
$MgSO_4 \cdot 7H_2O$	10.20 g
FeCitrate	2.75 g
KI	0.80 g
$MnSO_4$	0.50 g
$CuSO_4 \cdot 5H_2O$	0.30 g
$ZnCl_2$	0.25 g
Vitamin mixture	
Thiamine-HCL	200 mg
Riboflavin	200 mg
Pyridoxal-HCL	100 mg
Calcium-pantothenate	300 mg
Nicotinamide	2,000 mg
Folic acid	100 mg
	2,900 mg

measured by the method of Silber et al.[9] Proteins were determined by the Biuret method.[10]

Statistical analysis was carried out by applying unpaired student *t* test at 95% confidence.

Results

The levels of ascorbic acid decreased significantly in the plasma and liver subcellular fractions in scorbutic animals as compared with controls *(Table 2)*. No ascorbic acid could be detected in the nuclei and debri fraction.

The activity of butyrylcholinesterase also decreased significantly in all subcellular fractions of liver homogenate and in plasma *(Table 3)*.

Discussion

The activity of butyrylcholinesterase and ascorbic acid concentrations decreased significantly in the liver fractions and plasma as a result of feeding an ascorbic acid-deficient diet. Such decreases in liver butyrylcholinesterase activity have been observed in different forms of liver diseases, parenchymal diseases (hepatitis, cirrhosis) due to organophosphorus poisoning, myocardial infarctions,[11] and in nutritional deficiencies.[12] Although the liver and plasma butyrylcholinesterase has no known physiological function in humans, it has a considerable pharmacological significance because it is the enzyme that hydrolyzes the widely used

Table 2 Ascorbic acid levels in plasma and liver subcellular fractions of scorbutic guinea pigs

Fraction	Ascorbic acid (μg/mg protein)		
	Controls	Scorbutics	*P* value
Whole homogenate	0.375 ± 0.080	0.196 ± 0.078	< 0.010
Nuclei + debri	n.d.	n.d.	
Post mitochondrial supernatant	0.520 + 0.1600	0.020 + 0.002	< 0.001
Mitochondrial pellet	0.140 ± 0.110	0.036 ± 0.003	< 0.010
Plasma (μg/mL)	3.740 ± 1.420	1.090 ± 0.177	< 0.001

n.d., not detectable.
All values represent means ± SEM of six experiments.

Table 3 Butyrylcholinesterase activity in scorbutic guinea pigs

Fraction	Butyrylcholinesterase activity (nmoles/min/mg protein)		
	Controls	Scorbutics	*P* values
Whole homogenate	117.0 ± 13.4	75.0 ± 19.4	< 0.050
Nuclei + debris	n.d.	n.d.	
Post mitochondrial supernatant	470.3 ± 119.0	364.2 ± 124.4	< 0.050
Mitochondrial pellet	551.6 ± 92.4	399.5 ± 113.1	< 0.050
Plasma (nmoles/min/mL)	47.5 ± 10.1	24.4 ± 7.4	< 0.001

n.d., not detectable.
All values represent mean ± SEM of six experiments. Corticosterone values were 35.20 ± 17.5 and 193.33 ± 171.00 μg/100 mL of plasma in controls and scorbutic guinea pigs respectively ($P < 0.05$).

neuromuscular blocking agent succinylcholine-suxamethonium[13] and ester-type local anesthetics such as procaine-HCl or tetracaine-HCl.[14] Due to their brief action, these drugs are valuable in anesthesia, whereas other relaxants are excreted in urine and have a more prolonged effect. Significant decreases in butyrylcholinesterase may increase the intensity and duration of action of hydrolyzable muscle relaxants[15] and systemic toxicity of ester-type local anesthetic agents.[16] This could result in apnea of variable duration (suxamethonium apnea.)

Glucocorticoids are also known to decrease the activity of butyrylcholinesterase in various ways, by direct inhibition or stimulation of the synthesis of its biological inhibitor; or by interfering with the enzyme synthesis in the liver or increasing its rate of degradation. The levels of plasma corticosterone were increased significantly with the onset of scurvy in the present study. This increase could be attributed to the adrenal hypertrophy that accompanies scurvy. Stimulation of steroidogenesis in the early part of scurvy has also been shown.[17] However, this increase could contribute to the decrease in butyrylcholinesterase activity because glucocorticoids are known to inhibit the syntheses of nucleic acids and proteins in lymphoid tissues[18] and have been similarly shown to be capable of depressing the activity of butyrylcholinesterase, which is synthesized in the liver.[19]

The results in the present study could be significant to humans in that both these species (humans and guinea pig) cannot synthesize ascorbic acid and require it via dietary sources. The possibility should be considered that some rural populations in developing countries, which have been reported to have low leucocyte and blood ascorbic acid levels,[20,21] could show toxicity and sensitivity to drugs.

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