Effect of physical exercise on the specific activity of carbonic anhydrase isozyme in human erythrocytes

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Summary. Significant decreases in the levels of both carbonic anhydrase type B and total esterase activity of human erythrocytes were observed after physical exercise (bicycle ergometer, 150 W for 30 min). Since carbonic anhydrase B-dependent esterase activity likewise decreased, the decrease in the total esterase activity would be caused by the decrease of carbonic anhydrase B activity. The specific activity of carbonic anhydrase B tended to decrease after the exercise. On the other hand no such effects were noted for carbonic anhydrase type C.

Carbonic anhydrase (EC 4.2.1.1.) has 3 major types of isozymes, designated as type I (CA-B), type II (CA-C) and type III, in mammalian tissues. Immunologically these 3 types of isozymes do not show a cross-reaction with each other[1,2] and they are genetically controlled by different loci of the gene[2,3]. As a catalyst for the reversible hydration of CO_2, the enzyme plays an important role in the transport of CO_2 and acid/base equilibrium[4]. In erythrocytes an appreciable amount of CA-B and CA-C isozymes occur[2,4]. It has been reported that although the levels of CA-B in human erythrocytes vary considerably under certain pathological or physiological conditions[5,6], no significant changes occur in the levels of CA-C. In our recent study we found that the level of CA-B and also of total esterase activity decreases significantly after physical exercise[7]. The aim of the present study was to elucidate the changes in the specific activities of each isozyme after physical exercise. We describe the changes in CA-B-dependent esterase activity (active CA-B enzyme) and CA-C-dependent esterase activity (active CA-C enzyme) with muscular work, and compared them with the levels of total CA-B protein (active and inactive CA-B enzyme) and total CA-C protein (active and inactive CA-C enzyme), respectively.

Materials and methods. The subjects were 7 untrained healthy male volunteers (ages 20–22 years). They worked on a bicycle ergometer (Monark, Sweden) with a load of 150 W for 30 min. Heparinized blood samples were withdrawn from the cubital vein before and immediately after the exercise. The levels of CA-B and CA-C were assayed according to a single radial immunodiffusion technique[8] with a slight modification of the method described by Funakoshi and Deutsch[1]. The levels of CA-B and CA-C were expressed as mg enzyme per g hemoglobin. The esterase activity of the carbonic anhydrase was measured by a slight modification of the method of Armstrong et al.[9] with p-nitrophenyl acetate as a substrate. One unit of enzyme activity was expressed as 1 μmole of p-nitrophenol formed per min at 25 °C. The specific immunoadsorbent for CA-B was prepared by coupling Sepharose 4-B (Pharmacia Fine Chemicals, Sweden) with 20 mg of the anti-human CA-B horse IgG fraction according to the method described by Axen et al.[10] as described previously[11]. Total esterase activity and CA-C-dependent esterase activity (the activity after CA-B absorption) were determined according to the method described by Schapia et al.[12]. CA-B-dependent esterase activity was obtained as in the following equation: CA-B-dependent esterase activity (units/g Hb) = total esterase activity (units/g Hb) — activity after immunoadsorbent (units/g Hb). The specific activity (units/mg isozyme) of CA-B and CA-C was calculated by the following equations, respectively:

$$\frac{\text{CA-B-dependent esterase activity}}{\text{CA-B enzyme protein}} \quad \text{and}$$

$$\frac{\text{CA-C-dependent esterase activity}}{\text{CA-C enzyme protein}}$$

Results. As shown in the table, there were significant decreases in the levels of CA-B and total esterase activity after the 30 min of exercise, which is in keeping with the findings of our previous study[7] ($p < 0.05$ and $p < 0.02$, respectively). Likewise, the CA-B-dependent esterase activity showed a significant decrease ($p < 0.001$) and the specific activity of CA-B tended to decrease ($p < 0.1$). On the other hand no such effects were found for CA-C after the exercise.

Discussion. The activity of carbonic anhydrase isozymes is generally assayed by their dehydration and esterase activity as the total activity of carbonic anhydrase. Due to lability and the presence of endogenous inhibitor, the esterase activity or dehydration activity of carbonic anhydrase may not reflect the true amount of the enzyme protein. The immunochemical determinations of CA-B and CA-C isozymes by Funakoshi and Deutsch[1] reflect the total amount of the active and inactive enzyme protein. The steady-state level of an enzyme protein in general seems to be controlled by the rate of synthesis and the rate of degradation, and an alteration in either rate can affect the level of the enzyme[13]. However, matured erythrocytes do not synthesize enzyme proteins. Therefore, the level of an enzyme is considered to depend on the proteolytic degradation of enzyme proteins. Whether or not proteolytic activity will increase after physical exercise remains to be elucidated. The present study describes the decreases in the levels of CA-B, CA-B-dependent activity and the specific activity of CA-B under acute physical stress, whereas those of CA-C did not show significant changes. Therefore, the decrease in the total esterase activity would be caused by the decrease in CA-B-dependent activity. As shown in the table, the specific activity of CA-B is about 1/5 of that of CA-C in control erythrocytes in agreement with the findings of

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	Hematocrit (%)	Immunological level CA-B (mg/g Hb)	CA-C	Esterase activity Total activity (units/g Hb)	Dependent activity CA-B	CA-C	Specific activity CA-B (units/mg isozyme)	CA-C
Pre-exercise	44.8 ± 0.62	14.2 ± 1.53	1.7 ± 0.09	17.9 ± 1.69	11.0 ± 1.08	6.9 ± 0.62	0.81 ± 0.11	4.29 ± 0.47
Post-exercise	46.4 ± 0.70*	11.5 ± 0.87*	1.6 ± 0.09	15.0 ± 1.73**	8.0 ± 1.22***	7.0 ± 0.60	0.71 ± 0.12	4.46 ± 0.49

Values are expressed as mean ± SE. *$p < 0.05$, **$p < 0.02$, ***$p < 0.001$.

Gibbson and Edsall[14], and CA-B may contribute less to the total CO_2 hydrase activity; thus, the physiological meanings of the changes in CA-B levels remain to be elucidated. In normal subjects, inactive CA-B enzymes also seem to participate in the degradation process of the enzyme. There was a significant decrease in the total esterase activity in our previous study[7] and a decreasing tendency in the specific activity of CA-B in the present study, and one probable explanation for these results is that the active CA-B enzymes were converted in part to inactive enzymes with the exercise, possibly by binding to inhibitors or decreased zinc binding. Carbonic anhydrase has 1 atom of zinc per molecule of enzyme in its active center[4]. In an earlier study we found an inactive form of CA-B in the erythrocytes of children with primary renal tubular acidosis[15]. The addition of zinc chloride to hemolysates from these patients resulted in a marked increase in the activity of this enzyme. Quite recently we found that the zinc levels in erythrocytes vary during physical exercise (unpublished data). It was suggested that physical stress might have some effect on the affinity between CA-B enzyme protein and zinc. The simultaneous estimation of active and inactive enzyme in vivo provides useful data for physiological and experimental problems.

1 S. Funakoshi and H.F. Deutsch, J. biol. Chem. *245*, 2852 (1970).
2 R.E. Tashian and N.D. Carter, in: Advances in Human Genetics, vol. 7, p.l. Eds H. Harris and K. Hirschhorn. Plenum Press, New York 1976.
3 H.F. Deutsch, N. Taniguchi, S. Funakoshi and H. Hirai, Biochem. Genet. *6*, 255 (1972).
4 S. Lindskog, L.E. Henderson, K.K. Kannan, A. Liljas, P.O. Nyman and G. Strandberg, in: The Enzymes, vol. 5, p. 587. Ed. P.D. Boyer. Academic Press, New York 1971.
5 S. Funakoshi and H.F. Deutsch, J. Lab. clin. Med. *77*, 39 (1971).
6 N. Taniguchi, T. Kondo, N. Ishikawa, H. Ohno, E. Takakuwa and I. Matsuda, Analyt. Biochem. *72*, 144 (1976).
7 H. Ohno, N. Taniguchi, T. Kondo, T. Terayama, F. Hirata and T. Kawarabayashi, Int. J. Sports Med. *2*, 231 (1981).
8 G. Mancini, A.O. Garbonara and J.F. Heremans, Immunochemistry *2*, 235 (1965).
9 J.McD. Armstrong, D.V. Myers, J.A. Verpoorte and J.T. Edsall, J. biol. Chem. *241*, 5137 (1966).
10 R. Axen, J. Porath and S. Ernback, Nature *214*, 1302 (1967).
11 T. Kondo, N. Taniguchi, M. Murao and E. Takakuwa, Clinica chim. Acta *60*, 347 (1975).
12 E. Schapia, Y. Ben-Yoseph, F.G. Eyal and A. Russell, J. clin. Invest. *53*, 59 (1974).
13 R.T. Schimke and D. Doyle, A. Rev. Biochem. *39*, 929 (1970).
14 B.H. Gibbson and J.T. Edsall, J. biol. Chem. *239*, 2539 (1964).
15 T. Kondo, N. Taniguchi, K. Taniguchi, I. Matsuda and M. Murao, J. clin. Invest. *62*, 610 (1978).

Endotoxin inhibits the fluoride-stimulated adenylate cyclase activity of rat liver plasma membranes enriched with bile canaliculi

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Summary. Escherichia coli endotoxin inhibited the fluoride-stimulated adenylate cyclase activity of liver plasma membranes enriched with bile canaliculi. Inhibition was of a mixed competitive and uncompetitive type. This effect, which may result from changes of membrane organization, may have relevance in the understanding of endotoxin-cell membrane interactions.

Bacterial endotoxin (lipopolysaccharide, LPS) affects many aspects of hepatic functions[2]. Since LPS has high affinity for biological membranes[3] it may act by altering membrane configuration and membrane-bound enzyme activities, thereby triggering intracellular events[4]. In studying the mechanisms of LPS effect on the liver, we have previously found that the cholestatic effect induced by LPS[5,6] was paralleled by changes of membrane-bound Na^+,K^+-AT-Pase, which is involved in bile formation, while other membrane enzymes (i.e. Mg^{++}-ATPase and 5′-nucleotidase) were unaltered[7,8]. Since adenylate cyclase also plays a role in water and electrolyte transport[9], and may modulate LPS effects[4], we studied the effect of LPS on the activity of adenylate cyclase in preparations of rat liver plasma membranes (LPM) enriched with bile canaliculi.

Material and methods. Purified *Escherichia coli* 0127:B8 LPS, Boivin type, was purchased from Difco. ATP, phosphoenolpyruvate and pyruvate kinase were purchased from Boehringer; (α-^{32}P)ATP and (^{3}H)cyclic AMP from Amersham.

LPM enriched with bile canaliculi were prepared from male Sprague Dawley rats (250–300 g; Morini, Italy) as described by Boyer and Reno[10] with minor modifications[7]. LPM were suspended in the same buffer used for adenylate cyclase assay at concentration of 1–2 mg/ml and used the same day. Protein was determined by the method of Lowry et al.[11].

Adenylate cyclase activity was determined by measuring the conversion of ATP to cyclic AMP. Each sample (100 µl) contained 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM theophylline, 5 mM ATP containing 1.5 µCi of (α-^{32}P)ATP, 50 mM Tris-HCl buffer, pH 7.6. Phosphoenolpyruvate (5 mM) and pyruvate kinase (60 µg/ml) were used as ATP-regenerating system. LPS, when used, was dissolved in Tris buffer and added at concentrations from 20 to 200 µg/ml. The reaction was started by adding 40–100 µg of membrane proteins. The samples, in triplicate, were incubated for 10 min at 30 °C and the reaction was stopped by the addition of 0.1 ml of 0.5 M EDTA, pH 7.6, immediately followed by 3 min boiling. Then, 0.8 ml of cold 50 mM Tris-HCl buffer were added to the samples and they were centrifuged at 4 °C for 10 min. (^{3}H)Cyclic AMP (24 Ci/mM) equivalent to 30,000 cpm was added for the determination of recovery. The (^{32}P)cyclic AMP formed was isolated according to Salomon et al.[12]. Phosphodiesterase activity was measured using an incubation mixture identical to that for adenylate cyclase except that the ATP-regenerating system was omitted and the ATP was replaced with a trace amount of