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Identification of a specific protein in the mitochondrial fraction of the rat heart whose phosphorylation is inhibited by taurine

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Summary. It was previously reported that the mitochondrial fraction of the rat heart contained a specific protein with a molecular weight of approximately ~44 kDa whose phosphorylation was inhibited by taurine (Lombardini, 1994a). Isolation of the ~44 kDa phosphoprotein on a 1-dimensional polyacrylamide gel using traditional glycine buffers followed by re-electrophoresing the cut out proportion of the gel which corresponds to the ~44 kDa protein on a tricine-buffered gel resulted in sufficient pure protein for sequence analysis. The results indicate that the ~44 kDa phosphoprotein is pyruvate dehydrogenase.

Keywords: Amino acids – Taurine – Pyruvate dehydrogenase – Mitochondria – Rat heart – Phosphorylation

Introduction

In 1989 we first reported that taurine has an effect on the phosphorylation of specific proteins present in the rat heart (Lombardini and Liebowitz, 1989). In subsequent studies it was determined that rat heart mitochondrial preparations contained a specific protein with a molecular weight of approximately ~44 kDa whose phosphorylation was inhibited by taurine in a dose-dependent manner (Lombardini, 1992; 1994ab: 1996ab; Lombardini and Props, 1996ab). The concentration of taurine required to inhibit the phosphorylation by 50% was determined to be 9 mM, a concentration of taurine known to be present in the rat heart (Jacobsen and Smith, 1968).

However, since the identity of the mitochondrial ~44 kDa protein was previously unknown, the physiologic role of taurine in regulating the phosphorylation of this specific protein could not be assessed. Thus, these present studies in isolating and sequencing the ~44 kDa phosphoprotein were conducted to determine the identify of this unknown protein.

Materials and methods

Materials

Sprague-Dawley rats (200 g) were obtained from Sasco Inc. [γ-^{32}P]ATP (30 Ci/mmol) was purchased from New England Nuclear Corp. Taurine was purchased from Sigma Chemical Co. Tricine was purchased from Bio-Rad Laboratories.

Preparation of the mitochondrial subcellular fraction of the rat heart

Female Sprague-Dawley rats weighing 175–225 g were anesthetized with halothane, and the hearts were removed and placed on ice. The mitochondrial fraction of the rat hearts was prepared as previously described (Lombardini, 1994a). Five grams of rat hearts were washed in a Krebs-bicarbonate buffer (in mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.17, KH_2PO_4 1.2, and $NaHCO_3$ 23, pH 7.4, 2°C and then homogenized in a high potassium buffer (in mM): KCl 150, $MgSO_4$ 1.17, KH_2PO_4 1.2, and $NaHCO_3$ 25, pH 7.4 2°C with a Polytron homogenizer (6 s). The homogenate was centrifuged at 400 g (2,200 rpm) for 10 min to remove the cellular debris. The resulting supernatant was then centrifuged at 2,700 g (5,000 rpm) for 10 min to precipitate the mitochondria. The mitochondrial pellet was washed in the above high potassium buffer and recentrifuged at 2,700 g. The mitochondrial pellet was suspended in the high potassium buffer.

Phosphorylation assay

The phosphorylation assay was described previously (Lombardini, 1994a). The incubation mixture (0.25 ml) contained high potassium buffer (as already described), mitochondrial fraction (1.0 mg), and taurine when designated. After the mixture was preincubated for 2 min at 37°C, the reaction was initiated by adding 20 μCi [γ-^{32}P] ATP (10 μM). The reaction was allowed to continue for an additional 6 min, at which time 0.35 ml gel electrophoresis sample buffer [60 μM Tris-HCl; (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, 2 mM mercaptoethanol, and 0.00125% bromophenol blue] was added and the entire mixture was immediately boiled for 5 min.

Gel electrophoresis

Aliquots of the boiled incubation mixture were initially subjected to one-dimensional polyacrylamide gel electrophoresis (PAGE) on 12% gels according to the method of Laemmli (1970). After the electrophoretic run was stopped, the gels were washed with water (not stained), dried on a slab gel drier (Hoefer Pharmacia BioTech), and exposed to X-ray film (overnight, −80°C) to visualize the location of the ~44 kDa protein. The portion of the gel corresponding to the ~44 kDa protein was excised and the gel fragments were placed in the well of a tricine-buffered gel (13%T/6% C) and re-electrophoresed for 2 hrs at 30 volts (constant voltage) and then for 24 hours at 95 volts (Schägger and von Jagow, 1987). At the end of the run the gel was stained with Coomassie Brilliant Blue R, destained, dried, and exposed to X-ray film (overnight, −80°C). The radioactive band was then cut out of the gel and sent to the Protein & Nucleic Acid Facility at Stanford University, Stanford, CA., for digestion, HPLC separation of the peptides, and sequencing of two peptides.

Results and discussion

The effect of taurine (20 mM) on the phosphorylation of the ~44 kDa protein present in the mitochondrial fraction of the rat heart is shown in Fig. 1. In a

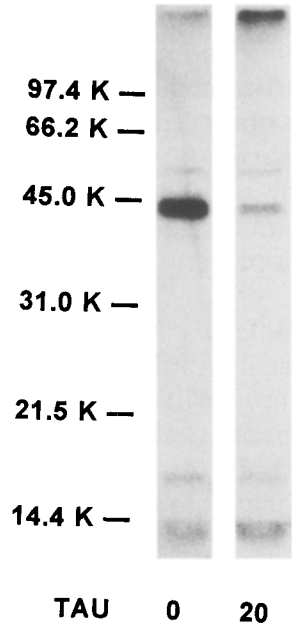

Fig. 1. Autoradiogram from a one-dimensional polyacrylamide gel electrophoresis experiment of the effect of taurine (20 mM) on the phosphorylation of an ~44 kDa protein present in the mitochondrial fraction of rat heart. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R, destained, dried, and exposed to X-ray film. Details of the phosphorylation assay and autoradiography are described in the Materials and methods section. *TAU* taurine

previous study (Lombardini, 1994a) it was determined that the concentration necessary to inhibit the phosphorylation of the ~44 kDa protein by 50% was 9.1 mM. A series of taurine analogues were also tested for their effects, both singly (Lombardini, 1994a) and in combination (Lombardini, 1996a) on the phosphorylation of this specific protein. In these structure-activity-relationship studies it was reported that certain taurine analogues were inhibitory while other analogues were stimulatory.

Isolation of the ~44 kDa phosphoprotein was achieved by first separating on a preparative (no wells) one-dimensional polyacrylamide gel and then re-electrophoresing the part of the gel containing the ~44 kDa phosphoprotein on an analytical tricine-buffered gel. The band of proteins obtained from the 1-D gel was further separated on a tricine-buffered gel into several additional bands, one of which contained radioactive phosphate. It was this portion of the tricine-buffered gel containing the phosphoprotein that was then cut out and sent to the Stanford Protein & Nucleic Acid Facility for digestion, HPLC separation of the various peptides, and sequence analysis. The sequence analysis of two peptides revealed that the isolated ~44 kDa phosphoprotein is pyruvate dehydrogenase.

Identification of the ~44 kDa phosphoprotein as pyruvate dehydrogenase appears reasonable since both the unknown phosphoprotein and pyruvate dehydrogenase have similar properties such as the following: 1) Pyruvate dehydrogenase, depending upon the source, has a molecular weight of

43.2 kDa in the mouse liver (Fitzgerald et al., 1992) and 41 kDa in the bovine kidney (Pettit, and Reed, 1982) and bovine heart (Reed and Yeaman, 1987). At the time that the rat heart mitochondrial protein whose phosphorylation was inhibited by taurine was observed (Lombardini, 1992) the designation of "~44 kDa" was only an approximation based on molecular weight standards. In this context, commercial pig heart pyruvate dehydrogenase migrates on 1-dimensional polyacrylamide gels in the same region as the rat heart radioactive ~44 kDa phosphoprotein. 2) Pyruvate dehydrogenase is a known phosphoprotein (Pettit et al., 1975). Obviously, the protein that we originally designated by its apparent molecular weight "~44 kDa" is a phosphoprotein as observed from the autoradiograph (Fig. 1). 3) We reported previously (Lombardini, 1994a) that the ~44 kDa protein is phosphorylated only in a serine residue(s) which is consistent with the reported serine phosphorylation sites for the mouse liver, mouse testis, human somatic, and human testis pyruvate dehydrogenases (Fitzgerald et al., 1992). 4) The phosphorylation of pyruvate dehydrogenase is cAMP independent (Technikova-Dobrova et al., 1993). This observation is also consistent with our data that cAMP has no effect on the phosphorylation of the ~44 kDa protein (Lombardini, 1992).

The pyruvate dehydrogenase complex converts pyruvate to acetyl CoA in a series of reactions important to aerobic energy metabolism in the cell. Pyruvate dehydrogenase complex, located in the matrix space of the mitochondria, is composed of pyruvate dehydrogenase, dihydrolipoyl dehydrogenase, and two regulatory enzymes, a kinase and a phosphatase, linked to a core component, dihydrolipoyl transacetylase (Reed et al., 1976). Pyruvate dehydrogenase is known to be regulated by phosphorylation which in turn is affected by numerous low molecular weight metabolites. For instance, the pyruvate dehydrogenase kinase is sensitive to fluctuations in the ratios of ATP/ADP, acetyl-CoA/CoA, and NADH/NAD and in the changes in the concentrations of Ca^{2+}, Mg^{2+}, K^{+} and pyruvate (Reed et al., 1976). The pyruvate dehydrogenase phosphatase is also sensitive to fluctuations in Mg^{2+}, Ca^{2+}, NADH (Reed et al., 1976) and polyamines, primarily spermine (Damuni et al., 1984).

While the physiologic concentrations of taurine in rat heart mitochondria have not been determined, there is information in the literature that mitochondria from various sources do indeed contain taurine. Immunocytochemical studies have demonstrated the presence of taurine in mitochondria of skeletal muscle (Terauchi and Nagata, 1993), hippocampus (Torp et al. 1991), and cerebellar cortex (Nagelhus et al., 1993).

Taurine has been reported to have significant effects on energy metabolism in cardiac tissue. Studies by Schaffer and colleagues (Schaffer et al., 1983) indicate that taurine affects myocardial metabolism via stimulation of glycolysis and aerobic metabolism. The rate of ATP biosynthesis in perfused rat hearts was increased by approximately 12% in the presence of 10 mM taurine. However, the authors concluded that the majority of this increase came from the effect of taurine on glycolysis. It was also reported (Lampson et al., 1983) that the levels of pyruvate and the ratio of ATP/ADP decreased in perfused rat hearts when exposed to 10 mM taurine. In other studies

(Harada et al., 1988) it was determined that taurine-depleted rat hearts demonstrated altered myocardial high energy phosphate metabolism due to elevated long chain acylcarnitine content and reduced long chain fatty acyl CoA levels. In this context, it is known that long chain fatty acyl CoA serves not only as a mitochondrial metabolite but also may alter adenine nucleotide translocase activity and energy metabolism (Shrago, 1978). Finally, it has been reported (Welty and Welty, 1981) that the mitochondrial Ca^{2+} ATPase activity in guinea pig hearts is inhibited by taurine which suggests that taurine may indirectly affect ATP levels via its influence on this enzyme.

Thus, the data presented in this study that taurine also inhibits the phosphorylation of pyruvate dehydrogenase implies an additional possibility that taurine has an effect on energy metabolism in the cell. These data are of significant interest in that taurine may be an additional effector of this enzyme or of the enzyme complex. Further studies are warranted to determine if taurine has a direct effect on either the kinase (inhibition) or the phosphatase (stimulation) associated with the pyruvate dehydrogenase complex.

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References

Damuni Z, Humphreys JS, Reed LJ (1984) Stimulation of pyruvate dehydrogenase phosphatase activity by polyamines. Biochem Biophys Res Commun 124: 95–99

Fitzgerald J, Hutchison WM, Dahl H-HM (1992) Isolation and characterization of the mouse pyruvate dehydrogenase E1α genes. Biochim Biophys Acta 1131: 83–90

Harada H, Allo S, Viyuoh N, Azuma J, Takahashi K, Schaffer SW (1988) Regulation of calcium transport in drug-induced taurine-depleted hearts. Biochim Biophys Acta 944: 273–278

Jacobsen JG, Smith LH Jr (1968) Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 48: 424–511

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680–685

Lampson WG, Kramer JH, Schaffer SW (1983) Potentiation of the actions of insulin by taurine. Can J Physiol Pharmacol 61: 457–463

Lombardini JB, Liebowitz SM (1989) Taurine modifies calcium ion uptake and protein phosphorylation in rat heart. In: Iwata H, Lombardini JB, Segawa T (eds) Taurine and the heart. Kluwer Academic Publishers, Massachusetts, pp 117–137

Lombardini JB (1992) Effects of taurine on protein phosphorylation in mammalian tissues. In: Lombardini JB, Schaffer SW, Azuma J (eds) Taurine: nutritional value and mechanisms of action. Plenum Press, New York (Adv Exp Med Biol 315: 309–318)

Lombardini JB (1994a) Inhibitory effects of taurine and taurine analogues on the phosphorylation of a ~44 K M_r protein present in a mitochondrial subfraction of the rat heart: partial characterization of the ~44 K M_r phosphoprotein. J Mol Cell Cardiol 26: 1675–1689

Lombardini JB (1994b) The inhibitory effects of taurine on protein phosphorylation: comparison of various characteristics of the taurine-affected phosphoproteins present

in rat retina, brain and heart. In: Michalk DV, Huxtable RJ (eds) Taurine in health and disease. Plenum Press, New York (Adv Exp Med Biol 359: 9–17)
Lombardini JB (1996a) Quantitative analysis of the combination dose-effects of taurine and taurine analogues on the phosphorylation of an ~44 kDa protein present in a mitochondrial subfraction of rat heart. J Cardiovas Pharm 28: 107–114
Lombardini JB (1996b) Taurine depletion in the intact animal stimulates *in vitro* phosphorylation of an ~44 kDa protein present in the mitochondrial fraction of the rat heart. J Mol Cell Cardiol 28: 1957–1961
Lombardini JB, Props C (1996a) Effects of benzophenanthridine alkaloids on the phosphorylation of an ~44 kDa protein present in a mitochondrial fraction of the rat heart. Biochem Pharm 51; 151–157
Lombardini JB, Props C (1996b) Effects of kinase inhibitors and taurine analogues on the phosphorylation of specific proteins in mitochondrial fractions of rat heart and retina. In: Huxtable R, Azuma J, Kuriyama K, Nakagawa M, Baba A (eds) Taurine 2: basic and clinical aspects. Plenum Press, New York (Adv Exp Med Biol 403: 343–350)
Nagelhus EA, Lehmann A, Ottersen OP (1993) Neuronal-glial exchange of taurine during hypo-osmotic stress: a combined immunocytochemical and biochemical analysis in rat cerebellar cortex. Neuroscience 54: 615–631
Pettit FH, Reed LJ (1982) Pyruvate dehydrogenase complex from bovine kidney and heart. Methods in Enzymology 89: 376–391
Pettit FH, Pelley JW, Reed LJ (1975) Regulation of pyruvate dehydrogenase kinase and phosphatase by acetyl-CoA/CoA and NADH/NAD ratios. Biochem Biophys Res Commun 65: 575–582
Reed LJ, Yeaman SJ (1987) Pyruvate dehydrogenase. The Enzymes 18: 77–95
Reed LJ, Pettit FH, Roche TE, Pelley JW, Butterworth PJ (1976) Structure and regulation of the mammalian pyruvate dehydrogenase complex. In: Shaltiel S (ed) Metabolic interconversion of enzymes. Springer, Berlin Heidelberg New York, pp 121–124
Schägger H, von Jagow G (1987) Tricine-sodium dodecyl sulfate – polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166: 368–379
Schaffer SW, Kramer JH, Lampson WG, Kulakowski E, Sakane Y (1983) Effect of taurine on myocardial metabolism: role of calmodulin. In: Kuriyama K, Huxtable RJ, Iwata H (eds) Sulfur amino acids: biochemical and clinical aspects. Alan R. Liss, New York (Prog Clin Biol Res 125: 39–50)
Shrago E (1978) The effect of long chain fatty acyl CoA esters on the adenine nucleotide translocase and myocardial metabolism. Life Sci 22: 1–6
Terauchi A, Nagata T (1993) Observation on incorporation of ^{3}H-taurine in mouse skeletal muscle cells by light and electron microscopic radioautography. Cell Mol Biol 39: 397–404
Torp R, Andiné P, Hagberg H, Karagülle T, Blackstad TW, Ottersen OP (1991) Cellular and subcellular redistribution of glutamate-, glutamine- and taurine-like immunoreactivities during forebrain ischemia: a semiquantitative electron microscopic study in rat hippocampus. Neuroscience 41: 433–447
Welty JD, Welty JC (1981) Effects of taurine on subcellular calcium dynamics in the normal and cardiomyopathic hamster heart. In: Schaffer SW, Baskin SI, Kocsis JJ (eds) The effects of taurine on excitable tissues. Spectrum Publications, New York, pp 295–312

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