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Serotonin modulates hepatic 6-phosphofructo-1-kinase in an insulin synergistic manner

Wagner Santos Coelho[a,b], Daniel Da Silva[a], Mônica Mesquita Marinho-Carvalho[a], Mauro Sola-Penna[a,*]

[a] Laboratório de Enzimologia e Controle do Metabolismo (LabECoM), Departamento de Fármacos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[b] Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

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ABSTRACT

Human and rat hepatic tissue express many serotonin (5-HT) receptor subtypes, such as 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_7 receptors, which mediate diverse effects. 5-HT is known to regulate several key aspects of liver biology including hepatic blood flow, innervations and wound healing. 5-HT is also known to enhance net glucose uptake during glucose infusion in fasted dogs, but little is known about the ability of 5-HT to control hepatic glucose metabolism, especially glycolysis. This study addresses the potential of 5-HT to regulate PFK activity and the mechanisms related to the enzyme activity. Based on our results, we are the first to provide evidence that 5-HT up-regulates PFK in mouse hepatic tissue. Activation of the enzyme occurs through the 5-HT_{2A} receptor and phospholipase C (PLC), resulting in PFK intracellular redistribution and favoring PFK association to the cytoskeletal f-actin-enriched fractions. Interestingly, 5-HT and insulin act in a synergistic manner, likely because of the ability of insulin to increase fructose-2,6-bisphosphate because the presence of this PFK allosteric regulator enhances the 5-HT effect on the enzyme activity. Together, these data demonstrate the ability of 5-HT to control hepatic glycolysis and present clues about the mechanisms involved in these processes, which may be important in understanding the action of 5-HT during the hepatic wound healing process.

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT[1]) is a biogenic amine that functions as a ligand for a large family of 5-HT receptors (Hoyer et al., 1994). Enterochromaffin cells of the gastrointestinal tract are responsible for the synthesis of 90% of the total 5-HT in the body (Ruddell et al., 2008). Serotonin is an important neurotransmitter within the central nervous system (CNS) and the autonomic nervous system (ANS), where the systems controlled by 5-HT are determined by the variable expression pattern of 5-HT receptors within the CNS and ANS (Hoyer et al., 1994). In the CNS, serotonin is known to control mood, behavior, learning, sleep and anxiety (Leysen, 2004; Harvey et al., 2004). Peripherally, serotonin is able to mediate vascular contraction and relaxation, gastrointestinal motility, cell proliferation, apoptosis and platelet aggregation (Roth et al., 1998).

Human hepatic metabolic functions are known to be regulated by both sympathetic (Cruise et al., 1987) and parasympathetic (Kato and Shimazu, 1983) branches of the ANS. Serotonergic nerve fibers are part of the peptidergic family of the ANS, localized in the tunica media on branches of the hepatic artery, portal vein, bile ducts and the connective tissue of the interlobular septa from humans (el-Salhy et al., 1993) and rats (Stoyanova, 2004). Serotonin presents the ability to elicit portal resistance and thereby regulate portal vein pressure (Richardson and Withrington, 1977; Cummings et al., 1986). The effects of serotonin on isolated mesenteric veins are blocked by ketanserin, a known 5-HT_{2A} antagonist, suggesting the involvement of the 5-HT_{2A} receptor (Cummings et al., 1986). The hepatic stellate cell (HSC) system, which is proposed to regulate hepatic blood flow, undergoes activation and acquires a smooth muscle cell-like phenotype with enhanced contractile capabilities in response to liver injury, has been demonstrated to express functional 5-HT_{2A} and 5-HT_{2B} receptors (Ruddell et al., 2006). Sinusoidal endothelial cells (SECs) also respond to a

Abbreviations: 5-HT, serotonin; 5-HT_{2A}, subtype 2A of the serotonin receptor; PI3K, phosphatidylinositol 3 kinase; PFK, 6-phosphofructo-1-kinase; F6P, fructose-6-phosphate; F1,6BP, fructose-1,6-bisphosphate; F2,6BP, fructose-2,6-bisphosphate; PKC, protein kinase C; PLC, phospholipase C.

* Corresponding author at: Laboratório de Enzimologia e Controle do Metabolismo (LabECoM), Departamento de Fármacos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro RJ, 21941-590, Brazil. Tel.: +55 21 2560 8438; fax: +55 21 2560 8438.
E-mail address: maurosp@ufrj.br (M. Sola-Penna).

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5-HT-mediated stimulus (Brauneis et al., 1992, Gatmaitan et al., 1996), which induces the activation of the myosin light chain through rapid influx of extracellular Ca^{2+} (Gatmaitan et al., 1996). 5-HT also inhibits cAMP synthesis and activates phospholipase A2 in these cells (Gatmaitan et al., 1996). In fact, many studies have demonstrated the responsiveness of the human hepatic vasculature to serotonin and ketanserin (Hadengue et al., 1987, 1989; Islam et al., 2000; Vorobioff et al., 1989), where serotonin plays a role promoting the contraction of the human hepatic artery (Islam et al., 2000).

The liver demonstrates a remarkable capacity for regeneration. Following a 70% partial hepatectomy (PHx), rodents are able to almost completely restore the lost mass within 14 days (Mabuchi et al., 2004). The process of wound healing is tightly regulated and is known to involve many hepatic and extra-hepatic tissues. In addition, recent studies highlighting the importance of serotonin in rodent hepatic wound healing after injury (Kulinskii et al., 1983a,b,c) led to increased interest in understanding the role of 5-HT in hepatic regeneration. It has been shown that serotonin elevates cAMP and cGMP within rat hepatocytes (Aslamova et al., 1985) and enhances hepatic poly-(A) RNA synthesis in normal healthy Wistar rats (Roy et al., 1985). Ketanserin has been demonstrated to be a potent inhibitor of liver regeneration and hepatocyte proliferation following PHx (Papadimas et al., 2006). Platelets have been identified as the major source of serotonin, which drives liver regeneration in a process that is mimicked by the 5-HT2 agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) and antagonized by ketanserin (Lesurtel et al., 2006). Again, these observations suggest a key role for the $5\text{-}HT_2$ family of receptors in mediating hepatic regeneration, likely through 5-HT mitogenic effects (Lesurtel et al., 2006; Hsu, 1995). However, the mechanisms underlying these observations are yet to be fully explored.

Serotonin acts within the liver, on the vessels supplying the liver with blood, and within the serotonergic nerve connections that regulate liver function (Ruddell et al., 2008). However, little is known about the actions of serotonin on carbohydrate metabolism in the liver. Moore and colleagues (Moore et al., 2004a,b, 2005b) conducted a series of studies and demonstrated enhanced hepatic glucose uptake after portal infusion of 5-HT or inhibitors of serotonin uptake. They further reported that fluvoxamine, a selective serotonin reuptake inhibitor, increases net hepatic glucose uptake, enhancing hepatic glycogen storage under condition of hyperglycemia and hyperinsulinemia, but not under euinsulinemic conditions (Moore et al., 2005a). Nevertheless, there have no studies that investigated the effects of serotonin upon hepatic glycolysis control.

In this study, we demonstrate that 5-HT up-regulates 6-phosphofructo-1-kinase (PFK; phosphofructokinase; ATP:D-fructose-6-phosphate-1-phosphotransferase; EC 2.7. 1.11), the rate-limiting enzyme of glycolysis, through the $5\text{-}HT_{2A}$ receptor by altering the enzyme cellular localization in an insulin synergistic manner.

2. Materials and methods

2.1. Materials

ATP, fructose-6-phosphate, ketanserin tartrate salt, fructose 2,6 bisphosphate (F-2,6-BP), insulin, serotonin, genistein, and wortmannin were purchased from Sigma Chemical St. Louis, MO, USA. U-73122, phorbol-12-myristate-13-acetate (PMA) was purchased from Calbiochem. ^{32}Pi was obtained from the Instituto de Pesquisas Energe ticas e Nucleares (SP). [γ-^{32}P]ATP was prepared according to (32a).

2.2. Mouse liver homogenate

Animal experimentation was conducted according to the Animal Care Procedures. Male Swiss mice (20–25 g) were fed *ad libitum* and killed by cervical dislocation with prompt removal of the liver.

Liver samples were weighed and homogenized for 30 s in a Polytron (Brinkmann Instruments, Westbury, NY, USA) in the presence of 6 vol of buffer containing 100 mM Tris–HCl, pH 7.4, 30 mM KF (potassium fluoride), 4 mM EDTA, 1.5 mM 2-mercaptoethanol and 250 mM sucrose, 1 mM sodium pyrophosphate (homogenizing buffer). Homogenized tissues were centrifuged for 10 min at $100 \times g$ at 4 °C to separate cellular debris and non-digested tissues. The resulting supernatant was used for all measured parameters.

The protein content measurements were performed according to (Bradford, 1976), and the total protein concentration used in each experiment was 50 μg/ml.

2.3. Preparation of soluble and bound PFK

Cytoskeleton-bound and soluble PFK were separated as described previously (El-Bacha et al., 2003; Gomes Alves and Sola-Penna, 2003). Tissues were homogenized as described above, and they were centrifuged for 10 min at $100 \times g$ at 4 °C to separate the cellular debris and the non-digested tissues. The resulting supernatant, which we named total homogenate (TH), was centrifuged for 30 min at $27{,}000 \times g$ at 4 °C. The resulting low-speed pellet, P1, is a fraction that is enriched with mitochondria, lysosomes, and nuclear components. The low-speed supernatant, S1, containing most of cytoplasmic structures was centrifuged for 30 min at $120{,}000 \times g$ at 4 °C. The resulting high-speed supernatant, S2, retains most of the soluble structures and the high-speed pellet, P2, contains the microsomal fraction and the cytoskeleton components as described previously (El-Bacha et al., 2003; Gomes Alves and Sola-Penna, 2003). All fractions (TH, S1, P1, S2 and P2) were assayed for PFK activity and protein concentration.

2.4. Radioassay for PFK activity

PFK activity was measured using the method described by (Sola-Penna et al., 2002), with the modifications introduced by (Zancan and Sola-Penna, 2005a) in medium containing 50 mM Tris–HCl (pH 7.4), 5 mM $(NH_4)_2SO_4$, 5 mM $MgCl_2$, and 1 mM [γ-^{32}P]ATP (4 μCi/μmol), 1 mM F6P was added after incubation in the appropriate conditions and length of time as indicated in the figures. The reaction was started with the addition of enough muscle homogenate to reach a final concentration of 100 μg protein/ml. The reaction was ended by the addition of a suspension of activated charcoal in 0.1 M HCl and 0.5 M mannitol. In addition, after centrifugation (10 min, $27{,}000 \times g$), the supernatant containing [1-^{32}P]fructose-1,6-bisphosphate was analyzed in a liquid scintillation counter. Appropriate blanks (samples without fructose-6-phosphate) were measured, and their values were subtracted from all measurements to account for any ATP hydrolysis. One unit of PFK was attributed to the formation rate of 1 nmol of fructose-1,6-bisphophate per minute.

3. Results

Incubation of mouse liver samples with 5-HT results in a significant upregulation of enzyme activity on TH in a dose-dependent manner (Fig. 1b). The maximum effect is observed with a dose of 10 μM 5-HT. At this dose, PFK activity was increased $50.13 \pm 8.91\%$ compared to control ($p < 0.05$, Student's *t*-test). We next incubated the liver homogenate with 10 μM 5-HT for 60 min, and aliquots were retrieved from the samples throughout the incubation at the