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Serotonin stimulates mouse skeletal muscle 6-phosphofructo-1-kinase through tyrosine-phosphorylation of the enzyme altering its intracellular localization

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Abstract

Serotonin (5-HT) is a hormone implicated in the regulation of many physiological and pathological events. One of its most intriguing properties is the ability to up-regulate mitosis. Moreover, it has been shown that 5-HT stimulate glucose uptake on skeletal muscle, suggesting that 5-HT may regulate glucose metabolism of peripheric tissues. Here we demonstrate that 5-HT stimulates skeletal muscle 6-phosphofructo-1-kinase (PFK) activity in a dose–response manner, through 5-HT_{2A} receptor subtype. Maximal activation of the enzyme (2.5-fold compared to control) is achieved in the presence of 25 pM 5-HT, increasing both PFK maximal velocity and affinity for the substrate fructose-6-phosphate. These effects occur due to tyrosine phosphorylation of the enzyme that is 2-fold enhanced upon 5-HT stimulation of skeletal muscles preparation. Once 5-HT-induced tyrosine phosphorylation of PFK is prevented by genistein, a tyrosine kinase inhibitor, the hormone stimulatory effect on PFK is abrogated. Wortmannin, a phosphatidylinositol-3-kinase (PI3K) inhibitor, does not interfere on 5-HT-induced stimulation of PFK, supporting that the observed effects are independent on insulin signaling pathway. Furthermore, 5-HT promotes the association of PFK to the muscle f-actin, suggesting that the hormone alters PFK intracellular distribution, favoring its association to the cytoskeleton. Altogether, our results support evidences that 5-HT augments skeletal muscle glucose consumption through stimulation of glycolysis key regulatory enzyme, PFK, throughout tyrosine phosphorylation and intracellular redistribution of the enzyme.

Keywords: Glycolysis; Phosphofructokinase; Regulation; Metabolism; Metabolom

Serotonin (5-hydroxytryptamine, 5-HT)[1] is a monoamine known for its neurotransmitter and vasoactive properties. It has also been implicated in the regulation of many others physiological and pathological events, including cellular growth and differentiation [1], regulation of blood glucose concentration [2], and a mitogenic effect in different cell lines [3–5]. Plasma 5-HT concentration increases in many pathological conditions such as non-insulin-dependent diabetes mellitus and hypertension [6]. Its diverse effects stems from the ability of the neurotransmitter to interact with multiple 5-HT receptors, classified as 5-HT_1 through 5-HT_7, with further subtypes within each class [7]. With the exception of the 5-HT_3 receptor which functions as a ligand-gated ion channel, all others subtypes belongs to a superfamily of G-protein coupled receptors that can modulate positively or negatively the adenylyl cyclase activity [8] and promote hydrolysis of phosphatidylinositol 4,5-biphosphate through activation of phospholipase C-β [9].

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[1] *Abbreviations used:* 5-HT, serotonin; 5-MeOT, 5-methoxytryptamine; 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.

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It has been shown that 5-HT enhances the glucose uptake on rat skeletal muscle through activation of 5-HT_{2A} receptors [10], which is present in this tissue [11]. This effect is due to the augmented expression and membrane content of glucose transporters GLUT1, GLUT3 and GLUT4, which is not dependent on IRS1, phosphatidylinositol-3-kinase (PI3K), nor the Akt (PKB) activity, indicating that these 5-HT effects are independent on insulin transduction signal pathway intermediates [10]. Indeed, these effects were attributed to the activation of Jak/STAT signaling pathway, previously described to be triggered by 5-HT via 5-HT_{2A} receptors in skeletal muscle [12]. The 5-HT_{2A} receptor is widely distributed in several organs, tissues and cells such as brain cortex [13], smooth muscle [14], fetal myoblasts [12] murine and human skeletal muscle [11], MCF-7 human breast cancer cell line [4] and human erythrocytes [15]. In erythrocytes, 5-HT was described to enhance the glycolytic flux through activation of 6-phosphofructo-1-kinase (PFK), which occurs through modulation of the enzyme binding to the membrane cytoskeleton [16].

6-Phosphofructo-1-kinase (PFK; phosphofructokinase; ATP:D-fructose-6-phosphate-1-phosphotransferase; EC 2.7.1.11), known as the main rate-limiting enzyme of glycolysis, has its activity correlated to the whole glycolytic pathway [17]. Many studies have reported the ability of PFK to associate with proteins of the cytoskeleton. The association to filamentous actin (f-actin) enhances the enzyme activity and its affinity to fructose-6-phosphate [3,18]. It has been shown that the phosphorylated form of PFK presents a higher affinity for f-actin than the dephosphorylated form of the enzyme [19,20]. Moreover, we have described that PFK association to cytoskeleton is favored by hormonal stimulus, such as β-adrenergic agonists [21] and insulin [22–24], through phosphorylation of the enzyme.

The current study reports the effects of 5-HT on mouse skeletal muscle PFK activity elucidating part of the mechanism of action. Our results support evidences that PFK participate on the mechanism by with 5-HT enhances skeletal muscle glucose consumption and suggest that the hormone regulates cell energy metabolism.

Materials and methods

Material

ATP, fructose-6-phosphate, ketanserin tartrate salt, genistein, wortmannin, 5-methoxytryptamine and spiperone were purchased from Sigma Chemical St. Louis, MO, USA. ^{32}Pi was obtained from Instituto de Pesquisas Energéticas e Nucleares (SP). [γ-^{32}P]ATP is prepared according to [25].

Mouse skeletal muscle homogenate

Animal experimentation was conducted accordingly to the Animal Care Procedures. Male Swiss Mice (20–25 g) fed *ad libitum* were killed by cervical dislocation and the muscles of hind legs were quickly removed and cleaned to remove fat and connective tissue. Muscle slices were weighted and homogenized for 30 s in Polytron (Brinkmann Instruments, Westbury, NY, USA) in the presence of 3 vol of a solution containing 30 mM KF, 4 mM EDTA, 15 mM 2-mercaptoethanol and 250 mM sucrose, pH 7.5 (homogenizing buffer).

All protein content measurement was performed according to [26], and the total protein concentration used in all experiments was 50 μg/ml.

Radioassay for PFK activity

PFK activity was measured by the method described by [27], with the modifications introduced by [23,24] in a medium containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$, 1 mM fructose-6-phosphate and 1 mM [γ-^{32}P]ATP (4 μCi/μmol). Reaction was started with the addition of muscle homogenate to reach a final concentration of 50 μg protein/ml. Reaction was stopped by addition of a suspension of activated charcoal in 0.1 M HCl and 0.5 M mannitol, and after centrifugation (10 min, 27,000*g*) the supernatant containing [1-^{32}P]fructose-1,6-bisphosphate formed was analyzed in a liquid scintillation counter. Appropriate blanks in the absence of fructose-6-phosphate were performed and subtracted from all measurements to discount the ATP hydrolysis. One unit of PFK was attributed to the formation rate of 1 nmol fructose-1,6-bisphophate per minute.

Spectrophotometric assay for PFK activity

PFK activity was assayed in a medium containing: 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$, 1 mM fructose-6-phosphate, 1 mM ATP, 0.5 mM NADH, 2 mU/ml aldolase, 2 mU/ml triosephosphate isomerase, 2 mU/ml α-glycerophosphate dehydrogenase and 50 μg/ml of protein in a final volume of 200 μl. Other reagents used are indicated for each experiment. Reaction was started by the addition of protein, and NADH oxidation was followed by measuring the decrease in absorbance at 340 nm in a microplate reader.

Total protein phosphorylation

In order to investigate the ability of 5-HT to induce protein phosphorylation in mouse skeletal muscle extracts, the homogenate was incubated with 25 pM 5-HT at 37 °C for 1 min in a medium containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$, and 1 mM [γ-^{32}P]ATP (60 μCi/μmol). The reaction was terminated with 0.1 vol 100% (p/v) trichlorloacetic acid and the samples were centrifuged (10 min, 3000*g*). The pellet was collected, washed with chloroform and centrifuged (10 min, 3000*g*). [^{32}P]phosphate incorporated in protein content was counted in a liquid scintillation counter.

Tissue fractionation

Tissue fractionation was performed after modifications of the protocol proposed in [21], based on actin-bound enzyme extraction protocols [19,20], as follows.

Homogenate was imposed to the tested conditions and was centrifuged for 10 min at 1250*g* (4 °C); the supernatant was collected and centrifuged for 30 min at 90,000*g* (4 °C). For the assays of enzymatic activity, the pellet was resuspended in 50 μl of the same homogenizing buffer and the PFK activity was measured as described above.

Western blotting

Western blotting assays were performed intended to asses the subcellular enzymatic localization. After tissue fractionation or incubation of homogenate with 25 pM 5-HT, where the reaction was stopped with 0.1 vol 100% (p/v) trichlorloacetic acid, samples were centrifuged for 10 min at 27,000*g* (4 °C). The pellet obtained for both situations was resuspended in 50 μl of sample buffer, and subjected to 8% SDS–

PAGE according to [28], transferred to nitrocellulose membrane, blocked with Tween–TBS (20 mM Tris–HCl, pH 7.5; 0.15 M NaCl; 0.1% Tween 20) containing 2% bovine serum albumin, and subsequently incubated with anti-actin (1:500) or anti-PFK (1:500) antiserum produced in mouse as reported previously [29]. The nitrocellulose membrane was washed five times with Tween–TBS, followed by 1-h incubation with goat anti-mouse conjugated with alkaline phosphatase (1:1000; Sigma Chemicals Co.). After that, the membranes were stained with alkaline phosphatase staining substrate. Membranes were scanned and analyzed using ImageQuant software (Molecular Dynamics, USA).

Immunoprecipitation

Mouse skeletal muscle homogenate were exposed to 25 pM 5-HT, at 37 °C, in presence or absence of genistein (26 μM), wortmannin (0.1 μM) or dimethylsulfoxide (DMSO) as the vehicle, for 1 min. Reaction was terminated by the addition of 0.1 ml SDS 10%, to a final volume medium of 1 ml. Samples were centrifuged for 5 min at 1250*g* (4 °C), and then supernatants were collected and incubated for 4 h at 4 °C, with anti-PFK (1:500)/protein A-agarose. The agarose beads were collected by centrifugation, washed three times with saline buffer and three times with SDS 10%, resuspended in 50 μl of sample buffer, boiled for 5 min. Samples were subjected to 8% SDS–PAGE according to [28], transferred to nitrocellulose membrane, blocked with Tween–TBS (20 mM Tris–HCl, pH 7.5; 0.15 M NaCl; 0,1% Tween 20) containing 2% bovine serum albumin, and subsequently incubated with monoclonal mouse anti-phosphotyrosine (1:500; Sigma Chemicals Co.), or monoclonal mouse anti-phosphoserine (1:500; Sigma Chemicals Co.). The nitrocellulose membrane was washed five times with Tween–TBS, followed by 1-h incubation with alkaline phosphatase-conjugated anti-mouse IgG (1:1000). After that, the membranes were stained with alcaline phosphatase staining substrate. Membranes were scanned and analyzed using ImageQuant software (Molecular Dynamics, USA). The same Western blots were reprobed with the antibody used for immunoprecipitation to ensure that equal amounts of protein were loaded in each lane.

Statistics and calculation

Statistical analysis was performed using the Sigma Plot/SigmaStat integrated software packages (Systat, CA, USA). Results are expressed as means ± SEM. Values for each group were compared by a paired and a non-paired Student's *t*-test, *P* values less than 0.05 was considered to indicate significance in all cases.

Enzyme kinetics parameters were calculated as described previously [22] by non-linear regression using the Sigma Plot/SigmaStat integrated software packages (Systat, CA, USA). Kinetic parameters for 5-HT activation of PFK activity in muscle extracts were achieved using the equation:

$$V = V_o + \frac{V_a \cdot [5-\mathrm{HT}]^n}{K_{1/2} + [5-\mathrm{HT}]^n} \quad (1)$$

where V is the calculated PFK activity at a given 5-HT concentration [5-HT], V_o is the PFK activity in the absence of 5-HT, V_a is the maximal activation observed, $K_{½}$ is the activation constant for 5-HT and n is the cooperativity index for activation.

Kinetic parameters for substrate (fructose-6-phosphate) activation were calculated using the equation:

$$V = \frac{V_{max} \cdot [\mathrm{F6P}]^n}{K_m + [\mathrm{F6P}]^n} \quad (2)$$

where V is the calculated PFK activity at a given fructose-6-phosphate concentration [F6P], V_{max} is the maximal velocity obtained at a saturating concentration of fructose-6-phosphate, K_m is the affinity constant for the substrate and n is the cooperativity index.

Results

Effects of 5-HT on mouse skeletal muscle PFK activity and kinetic parameters

The effects of 5-HT on PFK activity of mouse skeletal muscle were tested. Incubation of muscle extracts with different 5-HT concentrations significantly increases enzyme activity on total homogenate in a dose-dependent manner (Fig. 1). The maximal effect is achieved with 20–30 pM 5-HT where PFK activity raised from 31.2 ± 8.7 mU/mg protein in the control to 79.4 ± 4.9 mU/mg protein ($P < 0.05$, Student's *t*-test). This activation of PFK is sustained by higher concentrations of the hormone (up to 1 μM 5-HT, data not shown), and presents a $K_{½}$ for stimulation of PFK activity of 10.8 ± 1.7 pM with a cooperativity index of 2.0 ± 0.2, measured by non-linear regression as described under Materials and methods.

In order to understand the mechanism of PFK activation by 5-HT, we initially evaluated the kinetic properties of the enzyme upon hormonal stimulation. Our experiments reveal that 5-HT increases both the maximal velocity (V_{max}) and the affinity of PFK to fructose-6-phosphate, without any effect on the cooperativity index (n). Table 1 shows that upon stimulation of mouse muscle extracts with 25 pM 5-HT, V_{max} for PFK raises 43.9 ± 3.3 % ($P < 0.05$, Student's *t*-test), while the K_m for fructose-6-phosphate decreases 62.5 ± 5.2% ($P < 0.05$, Student's *t*-test). These effects are similar to those previously observed when PFK was phosphorylated and/or associated to the cytoskeleton [21,22], suggesting that these phenomena might be triggered upon stimulation of muscle by 5-HT.

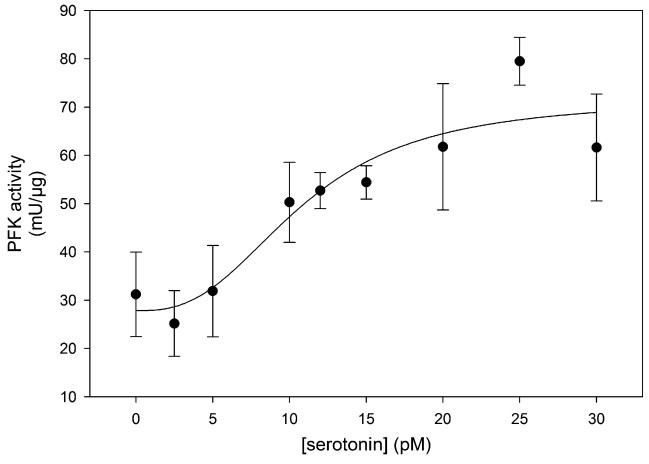

Fig. 1. Effects of 5-HT on PFK activity of mouse skeletal muscle. PFK activity was measured as described under Materials and methods. Assay was started by addition of muscle homogenates to a final concentration of 50 μg/ml. Reaction was stopped after 1 min reaction when the formation of product was linear (first-order kinetics). Plotted data are means ± standard errors of four independent experiments ($n = 4$). Solid line is the result of non-linear regression fitting Eq. (1) to the plotted values.

Table 1
Kinetic parameter for fructose-6-phosphate activation of PFK

Condition	V_{max} (mU/μg)	$K_{½}$ (mM)	n
Control	161.7 ± 7.6	0.24 ± 0.07	2.6 ± 0.8
25 pM 5-HT	232.7 ± 38.0[a]	0.09 ± 0.02[a]	2.9 ± 0.6

The parameters were calculated fitting the Eq. (2) described under Materials and methods to the experimental data obtained in an assay of PFK activity varying the concentrations of fructose-6-phosphate (0–3 mM). The values above are means ± standard errors of the parameters calculated for four independent experiments.

[a] Indicates that the value is statistically different from the one obtained in the control in the absence of serotonin ($P < 0.05$, Student's t-test).

Serotonin-induced PFK modulation is mediated through 5-HT$_{2A}$ receptor subtype

It has been shown that skeletal muscle expresses 5-HT$_{2A}$ receptor and that stimulation of this receptor modulates the glucose metabolism within these cells [10–12]. The stimulation of PFK activity by 5-HT is counteracted by the 5-HT$_{2A}$ receptor antagonist ketanserin in a dose-dependent manner, where 0.05 μM ketanserin partially reverts the effect, 0.1 μM ketanserin totally reverts the effect and 1 μM ketanserin lowers PFK activity below the control (Fig. 2a). The latter effect can be attributed to the reversal of an intrinsic activity of the receptor that is antagonized by the high concentration of ketanserin or to the antagonism of other 5-HT receptors, such as 5-HT$_{2B}$, 5-HT$_{2C}$ or 5-HT$_{1D}$, since the compound, at high concentrations can bind at these receptors, antagonizing their activities [30].

The effects of 5-HT are mimicked by 5-methoxytryptamine (5-MeOT), a 5-HT$_{2A}$ receptor agonist [30], corroborating that stimulation of PFK promoted by 5-HT occur through 5-HT$_{2A}$ receptor (Fig. 2b). Furthermore, stimulation of skeletal muscle PFK by 5-HT or 5-MeOT is antagonized by the 5-HT$_{2A}$ receptor antagonist, spiperone (Fig. 2b). The antagonism of spiperone is attenuated by increasing concentrations of 5-MeOT (Fig. 2b), supporting that these effects are mediated by the activation of 5-HT$_{2A}$ receptor. Moreover, it can be clearly observed that in the presence of 0.1 μM 5-MeOT and 1 μM spiperone, the PFK activity measured is lower that the control (Fig. 2b), as is observed in the presence of 1 μM ketanserin (Fig. 2a), supporting the hypotheses proposed above.

Signaling pathway involved in serotonin-induced PFK activation

Stimulation of mouse skeletal muscle with 25 pM 5-HT augments the ATP-dependent phosphorylation of total protein content (Fig. 3b, black bars), which occur in the same proportion of the formation of phosphotyrosine (Fig. 3b, gray bars). The formation of phosphotyrosine was analyzed through the quantification of the blot intensity using a monoclonal antibody against phosphotyrosine (anti-PY) in the band corresponding to 85 kDa, that is also

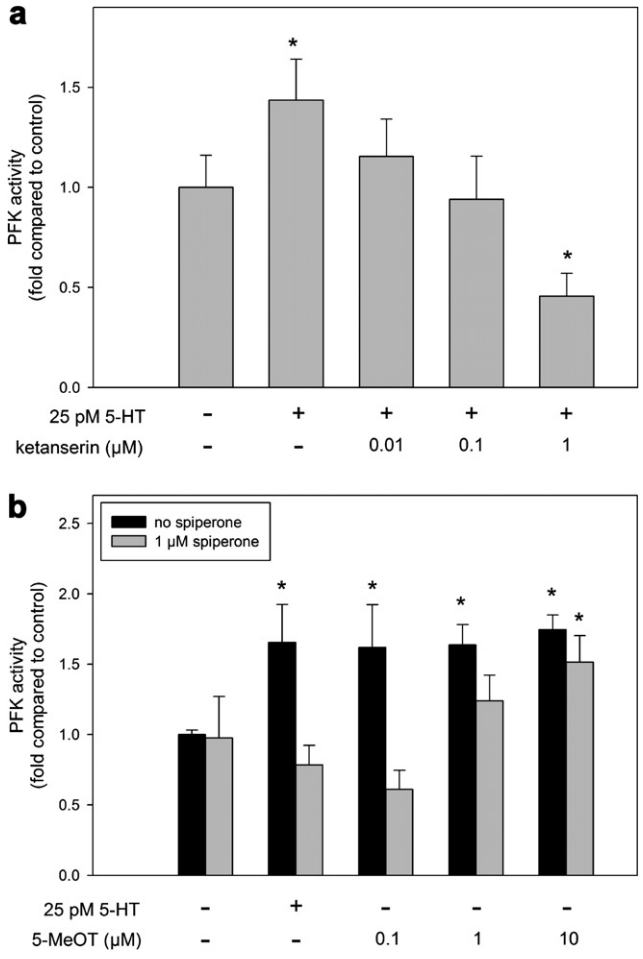

Fig. 2. 5-HT effects on mouse skeletal muscle PFK occur through 5-HT$_{2A}$ receptor. Mice muscle homogenates were assayed for PFK activity in the absence and presence of 5-HT$_{2A}$ receptor agonists and antagonists. (a) PFK activity was measured in the absence or the presence of 25 pM 5-HT and the indicated concentrations of genistein. PFK activity is expressed as fold of control activity, in the absence of additives. *Indicates statistical difference comparing to the control ($P < 0.05$, Student's t-test). (b) PFK activity was measured in the absence or the presence of 25 pM 5-HT, 1 μM spiperone and the indicated concentrations of 5-MeOT. PFK activity is expressed as fold of control activity, in the absence of additives. *Indicates statistical difference comparing to the respective control (black or gray bars) in the absence of 5-HT of 5-MeOT ($P < 0.05$, Student's t-test).

recognized by PFK antibody (anti-PFK), as shown in the representative Western blot analysis (Fig. 3a).

In order to prove that 5-HT induces the tyrosine phosphorylation of PFK, mouse skeletal muscles were incubated in the presence or not of 25 pM 5-HT, 26 μM genistein, an inhibitor of tyrosine kinases, and 0.1 μM wortmannin, the inhibitor of PI3K, a serine/threonine kinase stimulated upon insulin signaling. After incubation, these samples were immunoprecipitated using the anti-PFK, submitted to SDS–PAGE, transferred and blotted using anti-PY or a monoclonal antibody against phosphoserine (anti-PS). A representative Western blot is presented in Fig. 3c. The quantification of distinct experiments reveals that 5-HT promote the phosphorylation of PFK in tyrosine residues, an effect that is prevented by the

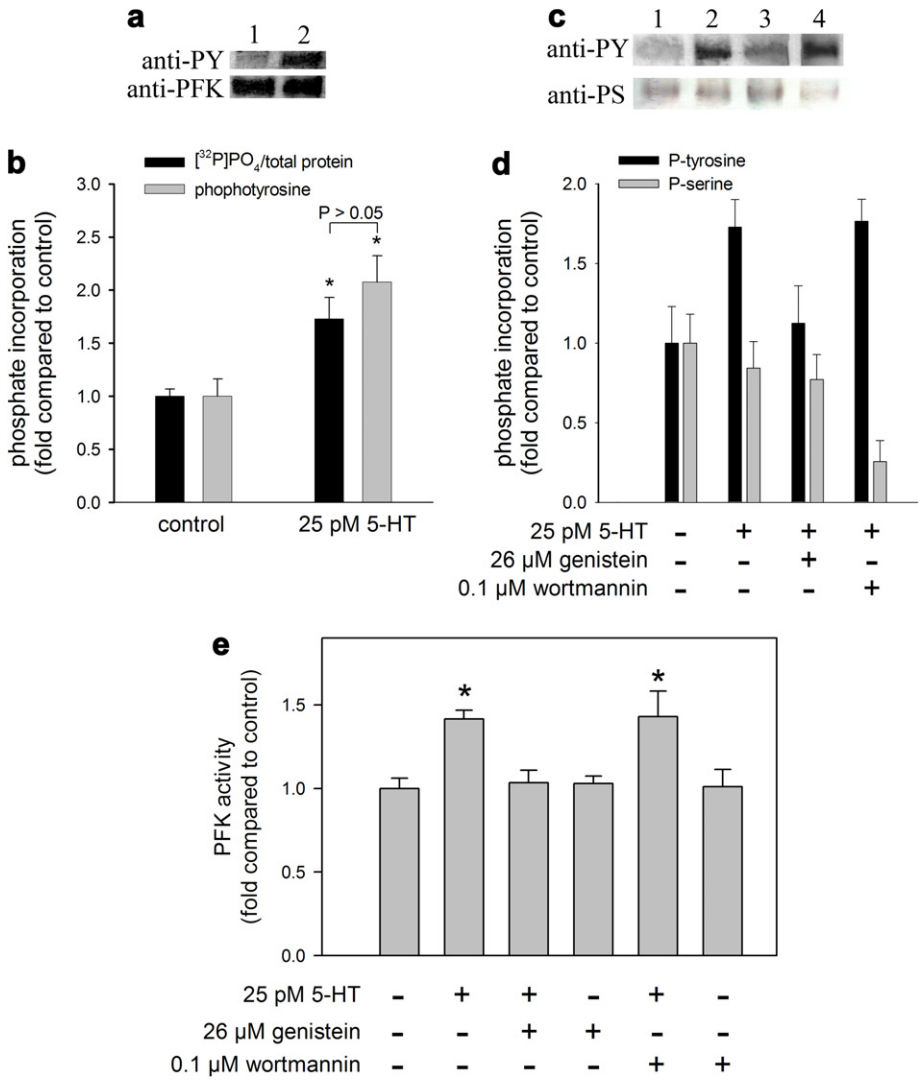

Fig. 3. Effects of 5-HT on PFK phosphorylation and activity. (a and b) Mouse skeletal muscle homogenates were incubated in the absence (control) or in the presence of 25 pM 5-HT for 1 min and assayed for total protein phosphorylation or tyrosine phosphorylation of PFK as described under Materials and methods. (a) Representative Western blot of control (lane 1) and 25 pM 5-HT samples (lane 2) submitted to SDS–PAGE and probed with monoclonal antibody against phosphotyrosine (anti-PY) or against PFK (anti-PFK). (b) Relative amount (compared to control) of phosphate incorporation on total protein (black bars) or of tyrosine phosphate on PFK (gray bars). Values are means ± standard errors of three independent experiments ($n = 3$) *Indicates statistical differences comparing to the control ($P < 0.05$, Student's t-test). (c and d) Mouse skeletal muscle homogenates were incubated in the absence or the presence of 25 pM 5-HT, 26 μM genistein and 0.1 μM wortmannin, then were immunoprecipitated using an anti-PFK antibody. The pellets were resuspended and submitted to a Western blotting using anti-PY or anti-PS monoclonal antibodies. (c) A representative blot of the control (lane 1), 25 pM 5-HT (lane 2), 25 pM 5-HT + 26 μM genistein (lane 3) and 25 pM 5-HT + 0.1 μM wortmannin (lane 4). (d) Quantification of the Western blot experiments using anti-PY and anti-PS ($n = 3$). (e) PFK activity of the samples submitted to the same treatment set above. PFK activity is expressed as fold of control activity, in the absence of additives. *Indicates statistical difference comparing to the control ($P < 0.05$, Student's t-test).

presence of the tyrosine kinase inhibitor, genistein, but not by wortmannin (Fig. 3d, black bars). On the other hand, neither 5-HT nor genistein alters the amount of phosphoserine in PFK, which is reduced upon incubation with wortmannin (Fig. 3d, gray bars). These data indicate that 5-HT induces the phosphorylation of tyrosine, but not serine, residues of PFK due to the activation of a tyrosine kinase, through a pathway independent on the insulin signaling pathway.

This statement is supported by the fact that phosphorylation of PFK tyrosine residues is responsible for the stimulation of the enzyme promoted by 5-HT. Fig. 3e shows the effects of genistein and wortmannin on the 5-HT-induced activation of PFK. It can be seen that genistein reverts the effects of 5-HT on the enzyme, which is not altered by wortmannin. These results directly correlate the effects of 5-HT on the phosphorylation and activation of the enzyme, strongly suggesting that the later effects depends on the former.

Effects of 5-HT on PFK intracellular localization

As previously reported in our laboratory [21–24] phosphorylated forms of PFK presents a higher affinity for

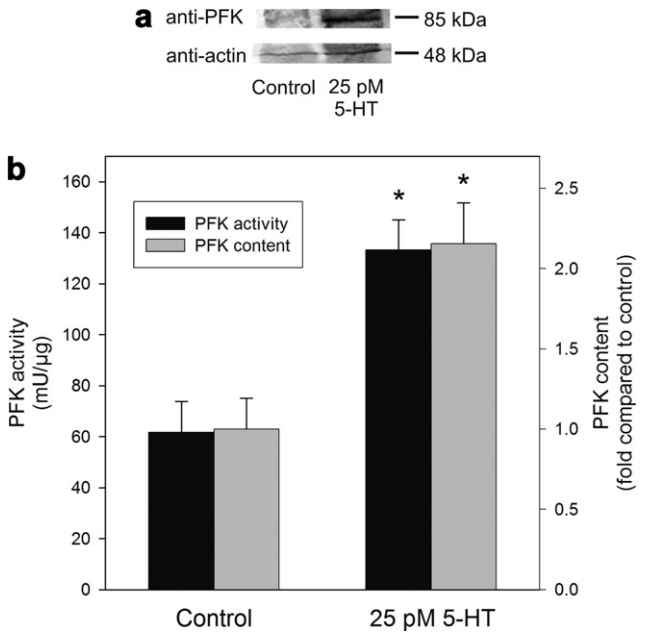

Fig. 4. Effects of 5-HT on PFK cellular localization. Mouse skeletal muscle homogenates were incubated in the absence (control) or in the presence of 25 pM 5-HT for 1 min, subjected to ultracentrifugation (30 min, 100,000*g*) and pellets were analyzed for PFK activity and content as described under Materials and methods. (a) Representative Western blot of pellets subjected to SDS–PAGE and probed with monoclonal antibody against PFK (anti-PFK) or actin (anti-actin). (b) Black bars: PFK activity in the pellets measured as described under Materials and methods. Gray bars, relative amount of PFK (compared to control) measured by Western blotting analyzes as described under Materials and methods. *Indicates statistical differences comparing to the control ($P < 0.05$, Student's *t*-test).

actin filaments, which favors its activity. Aimed at assessing whether 5-HT modulates PFK binding to f-actin filaments, we fractionated skeletal muscle homogenate and measured the effects of 5-HT on the PFK activity and content in the f-actin enriched fraction as described in Materials and methods (Fig. 4b). As it can be seen, 5-HT increases $116 \pm 14\%$ the enzyme activity and $115 \pm 18\%$ the PFK content in the f-actin enriched fraction when compared to controls ($P < 0.05$, Student's *t*-test). A representative Western blotting of the co-sedimentation experiment is shown (Fig. 4a).

Discussion

Serotonin has been described to activate glucose transport in murine skeletal muscle via tyrosine kinase activity of the Jak/STAT signaling pathway [12]. However these authors did not show whether this effect occurs concurrently to the enhancement of glucose metabolism. Our results demonstrate that 5-HT stimulates PFK activity through tyrosine phosphorylation of the enzyme enhancing its association to f-actin. PFK is the key regulatory enzyme of glycolysis and its activity is correlated to the whole glycolytic flux [3,17,19–24]. In this context, PFK phosphorylation and association to f-actin are important mechanisms of this enzyme regulation, involved in many glycolytic stimulatory signals [16,19–24,29,31–34]. Our work shows that these mechanisms are involved in the 5-HT effects on PFK, suggesting that this hormone stimulates skeletal muscle glycolysis.

The observed effects of 5-HT occur through activation of 5-HT_{2A} receptor, being mimicked by this receptor agonist 5-MeOT and abrogated by ketanserin and spiperone, two of its antagonists. 5-HT_{2A} receptor subtype co-immunoprecipitate with Jak/STAT that presents tyrosine kinase activity [12], suggesting the physical interaction between these proteins. This fact elects Jak/STAT system as a candidate to phosphorylated PFK upon 5-HT stimulus. The tyrosine kinases triggered by insulin signaling are discarded since 5-HT effects are not influenced by wortmannin. These results are in accordance with previous work, which have demonstrated that the increased glucose uptake mediated by serotonin through 5-HT_{2A} happens in a way independent on IRS1, PI3K, or Akt, all known insulin signaling intermediates [11]. Moreover, our results show that 5-HT promotes the association of PFK to f-actin, an effect observed upon non-serotonin-induced tyrosine phosphorylation of the enzyme [22,24]. This association occur concurrently to the same kinetic alterations presented in Table 1 [22], suggesting that the molecular mechanism by which 5-HT modulates PFK is shared by other signals.

In conclusion, our results show that serotonin up regulate the glycolytic flux, through activation of PFK catalytic activity. The enzyme stimulation is dependent on tyrosine-phosphorylation, followed either by kinetics alterations and cellular relocation of the enzyme, which in part, may also alter the same kinetic parameters. Furthermore, the results described here occur mediated by 5-HT_{2A} receptor subtype, in a non-insulin-dependent pathway. These results might be elucidative to other metabolism 5-HT effects.

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