A Novel Mechanism for Regulating Hepatic Glycogen Synthesis Involving Serotonin and Cyclin-Dependent Kinase-5

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Hepatic autonomic nerves regulate postprandial hepatic glucose uptake but the signaling pathways remain unknown. We tested the hypothesis that serotonin (5-hydroxytryptamine [5-HT]) exerts stimulatory and inhibitory effects on hepatic glucose disposal. Ligands of diverse 5-HT receptors were used to identify signaling pathway(s) regulating glucose metabolism in hepatocytes. 5-HT had stimulatory and inhibitory effects on glycogen synthesis in hepatocytes mediated by 5-HT1A and 5-HT2B receptors, respectively. Agonists of 5-HT1A receptors lowered blood glucose and increased hepatic glycogen after oral glucose loading, and stimulated glycogen synthesis in freshly isolated hepatocytes with greater efficacy than 5-HT. This effect was blocked by olanzapine, an antagonist of 5-HT1A receptors. It was mediated by activation of phosphorylase phosphatase, inactivation of glycogen phosphorylase, and activation of glycogen synthase. Unlike insulin action, it was not associated with stimulation of glycogenolysis and was counterregulated by cyclin-dependent kinase (cdk6) inhibitors. A role for cdk5 was supported by adaptive changes in the coactivator protein p35 and by elevated glycogen synthesis during overexpression of p35/cdk5. These results support a novel mechanism for serotonin stimulation of hepatic glycogenesis involving cdk5. The opposing effects of serotonin, mediated by distinct 5-HT receptors, could explain why drugs targeting serotonin function can cause either diabetes or hypoglycemia in humans.

The stimulation of hepatic glucose uptake after oral glucose ingestion or after glucose infusion into the portal vein cannot be fully explained by hyperglycemia, hyperinsulinemia, and hyperglucagonemia (1–4). A mechanism involving hepatic nerves, described as the portal signal, has a major role in postprandial hepatic glucose disposal (5,6). It involves transmission of an afferent signal from a glucose sensor in the portal vein to the central nervous system that is transduced to an efferent response to the liver involving inhibition of sympathetic (noradrenergic) nerves and activation of parasympathetic (cholinergic) nerves (3,4,7–9). This is supported by stimulation of hepatic glycogen synthesis and glycogen synthase during activation of the hepatic vagal nerve (7). Studies on isolated hepatocytes and perfused liver support a catabolic role for norepinephrine in promoting glycogenolysis by activation of glycogen phosphorylase (7–9) but not a major anabolic role for acetylcholine on glycogen synthesis (10–14). Infusion of acetylcholine into the portal vein or hepatic artery promotes either hepatic glucose uptake or production (10–13). The former effect was mimicked by choline (12) and the latter was attributed to release of nonadrenergic neurotransmitters (13). Human liver is richly innervated with serotoninergic nerves (15,16), and infusion of serotonin into the portal vein of dogs stimulates hepatic glucose uptake (17), making serotonin a potential candidate for regulating hepatic glucose disposal.

Serotonin (5-hydroxytryptamine [5-HT]) is a neurotransmitter in the central nervous system and peripheral nerves. It is involved in various physiological functions, including synaptic transmission, gastrointestinal motility, and cardiovascular control. Serotonin is known to act on the liver, where it plays a role in regulating glucose metabolism. The study by Tudhope et al. (1) investigated the role of serotonin in hepatic glycogen synthesis, proposing a novel mechanism involving serotonergic signaling pathways.

**Hepatocyte Isolation**

Hepatocytes were isolated from male Wistar rats fed ad libitum (200–300 g body wt) obtained from B&K, Hull or Harlan, Bicester, U.K. They were suspended in minimum essential medium (MEM) containing 5% newborn calf serum and seeded on gelatin-coated (1 mg/mL) plates (20). Unless otherwise indicated (Fig. 1A and Fig. 7), incubations for metabolic studies were started after a 2–3-h culture to allow cell attachment. For experiments involving transfection with cyclin-dependent kinase-5 (cdk5), p35, or protein phosphatase-1 (PP1) adenoviral vectors (Vector Biolabs, Philadelphia, PA), the serum-containing medium was replaced after 2 h with...
FIG. 1. Effects of 5-HT receptor agonists on glycogen synthesis in vitro or in vivo. A: Glycogen synthesis determined during a 3-h incubation without or with 50 nmol/L insulin or 100 μmol/L 5-HT in hepatocytes precultured for 2 or 24 h, expressed as percentage of respective control. Basal rates of glycogen synthesis (nmol/3 h/mg) were 8.1 ± 1.9 (n = 6) for 2 h and 17.6 ± 3.5 (n = 5) for 24 h culture. *P < 0.05; **P < 0.01 vs. control; #P < 0.05 vs. insulin. B–F: Glycogen synthesis (% control) in hepatocytes precultured for 2 h. B: Effects of 5-HT concentration plotted as linear or log (inset) scale; n = 5–8. *P < 0.05 vs. control. C: Effects of agonists: methyl-5HT (MSHT), 8-hydroxy-2-(di-n-propylamino)tetralin (5-HT1A agonist), and CP-93129 (5-HT1B agonist). D: Effects of antagonists of 5-HT1A (S-WAY100135, 10 μmol/L), 5-HT1B (SB-224289, 10 μmol/L), 5-HT2A (ketanserin, 10 μmol/L), and 5-HT2B/C (SB-206553, 2 μmol/L) receptors (5HTR) on the stimulation by 100 μmol/L 5-HT; n = 4–6. E: Combined effects of olanzapine and 5-HT (100 μmol/L); n = 4. #P < 0.05 vs. 5-HT alone. *P < 0.05 effect of olanzapine. F: Combined effects of
serum-free MEM containing vectors. After 2 h, the medium was replaced with MEM containing 10 mM dexamethasone and 5 mM glucose, and the hepatocytes were cultured for 18 h before the metabolic studies (20).

**Oral glucose tolerance.** The protocol was approved by the local Animal Use and Care Committee in accordance with the Home Office Guidance (Scientific Procedures Act 1986) and the National Research Council Guide for Care and Use of Laboratory Animals. Male Wistar rats (Harlan, Bicester, U.K.) were fasted for 18 h and then injected intraperitoneally with vehicle (10% DMSO/90% saline) without or with agonist. After 15 min, a blood sample was collected (time zero) and glucose (2 g/kg body wt) was given by gavage. Blood glucose was determined at the time intervals indicated (Accuchek Aviva; Roche, Lewes, East Sussex, U.K.). The liver was removed at 120 min, and glycogen was determined as previously described (23).

**Metabolic studies on hepatocytes.** Glycogen synthesis was determined from 3-h incubations in MEM containing 15 mM glucose and [U-14C]glucose (2 μCi/mL) (20), and glycolysis from 1- or 3-h incubations in medium containing 15 mM glucose and [3-3H]glucose (1 μCi/mL) (20). Rates of both glycogen synthesis and glycolysis were linear during 3 h. Glycogen synthesis is expressed as nanomoles of glucose incorporated per 3 h per milligram protein, unless otherwise indicated. Freshly isolated hepatocytes have more variable rates of glycogen synthesis (in basal and stimulated conditions) than 24 h cultured hepatocytes, and for data sets comparing agonists or antagonists, results are expressed as percentage of either the basal rate or the maximal stimulation as indicated.

**Enzyme activity and metabolite determination.** Hepatocytes were snap-frozen in liquid nitrogen and stored at −80°C. For determination of GPa (phosphorylated form of glycogen phosphorylase) activity, the cells were extracted in 150 mM KF (potassium fluoride), 25 mM HEPES, 0.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and benzamidine, pH 7.4. GPa activity was determined as previously described (24). Phosphorylase phosphatase was determined by modification of a previously described method (25). Hepatocytes were extracted in the above buffer without KF, and lysates were incubated at 30°C. Samples were removed at 5-min intervals (up to 20 min) and quenched with an equal volume of extraction buffer containing 300 mM KF. GPa activity was determined (24) in the 13,000-g supernatant. Glycogen synthase was determined as previously described (24). Enzyme activities are expressed as milliunits per milligram of protein, where 1 nU is the amount of enzyme catalyzing 1 mol substrate per minute. Fructose 2,6-bisphosphate (fructose 2,6-P2) was determined as previously described (26).

**Immunoblotting.** The cells were extracted in gel loading buffer (30 mM Tris pH 6.8, 0.5% SDS, 7.5% glycerol, 0.01% bromophenol blue, 1% v/v mercaptoethanol) resolved by SDS-PAGE, and the protein was transferred to nitrocellulose. Membranes were blocked either with 3% (w/v) nonfat dry milk and probed with antibodies against cdk5 and p35 (C-8:sc-173 and C19:sc-820, 1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) or with 5% BSA and probed with antibodies against phosphoprotein: GPa-Sc14 (27), phosphofructokinase-2 (PFK2)/fructose bisphosphatase-2 Ser32 (26), and PIIa-Thr32 or PIIe (2581 and 2582; New England Biolabs). Proteins were detected using peroxidase-conjugated anti-IgG and enhanced chemiluminescence (Amersham).

**mRNA analysis.** RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA) and treated with DNaseI (Roche), and cDNA was synthesized from 1 μg total RNA with random hexamers and Superscript III (Invitrogen). Real-time RT-PCR for 5-HT1 (nonexon spanning) and 5-HT2 receptors, respectively. SYBR green (Rn99999916_s1) and cyclophilin (Rn00690933_m1) were used as internal reference (28). mRNA levels for 5-HT receptors were determined at 2- and 24-h culture. GAPDH and cyclophilin mRNA levels (normalized to total RNA) were the same at 2 and 24 h (GAPDH, 1.03 ± 0.13; cyclophilin, 1.14 ± 0.11; 24/2 h; n = 10). However, expression of the 5-HT2A receptor declined by 70% at 24 h (0.32 ± 0.05; n = 10; P < 0.00001) whereas expression of 5-HT1B, 5-HT2B, and 5-HT6 receptors increased three- to fivefold at 24 h relative to 2 h (5-HT1B, 4.78 ± 0.71, n = 10, P < 0.0003; 5-HT2B 2.7 ± 0.3, n = 14, P < 0.0003; 5-HT6, 3.37 ± 0.28, n = 6, P < 0.03). Expression of other 5-HT receptors was either unchanged or below detection limits. Because of the changes in 5-HT receptor expression that occur during 24-h culture, in the rest of this study, hepatocytes were used 2-3 h after plating except for Fig. 7, which involved 24-h culture to allow protein expression.

**Stimulatory and inhibitory effects of 5-HT receptor agonists and antagonists on glycogen synthesis.** We used agonists (Fig. 1C) and antagonists (Fig. 1D) of 5-HT1 and 5-HT2 receptors (29) to identify receptors that regulate glycogenesis. Methyl-5HT, a mixed agonist of 5-HT1 and 5-HT2A receptors (30), and agonists of 5-HT1A (7-hydroxy-2-dipropylaminotetralin hydrobromide) and 5-HT1B (CP-93129) receptors caused greater stimulation than 5-HT (Fig. 1C). However, agonists of 5-HT1D (1-694247 and GR-46611) and 5-HT2C (R[1]-2,5-dimethoxy-4-iodoamphetamine) receptors were ineffective (not shown), and m-chlorophenylpiperazine (mCPP), an agonist of 5-HT2B and 5-HT2C receptors, inhibited (1 μmol/L mCPP, 75 ± 2%; 10 μmol/L mCPP, 72 ± 2%; n = 4; P < 0.005). The stimulation by 5-HT (100 μmol/L) was significantly enhanced with antagonists of 5-HT1A, 5-HT1B, and 5-HT2A receptors, but it was significantly enhanced with SB-20553, an antagonist of 5-HT2C receptors (Fig. 1D), indicating a 5-HT-mediated inhibitory mechanism that is sensitive to SB-206553. The stimulation by 5-HT was inhibited with olanzapine (Fig. 1E), an antipsychotic drug that binds to 5-HT1B and 5-HT2A receptors (31,32), further supporting involvement of these receptors. Stimulation by methyl-5HT was counteracted with antagonists of 5-HT1B, 5-HT1A, and 5-HT2A receptors and olanzapine (Fig. 1F), unlike 5-HT stimulation (Fig. 1D), it was not further enhanced by SB-20553 (Fig. 1F), indicating that methyl-5HT activates the stimulatory but not inhibitory receptors. When the effects of methyl-5HT were tested in vivo (5 μmol/kg) during an oral glucose tolerance test, the agonist lowered blood glucose (Fig. 1G) and enhanced glycogen storage (Fig. 1H), consistent with the stimulation of glycogen

antagonists (as in D and 30 μmol/L olanzapine [OLZ]) and methyl-5HT (10 μmol/L); n = 4–6. *P < 0.05 vs. methyl-5HT alone. Time course of blood glucose concentration (G) after an oral glucose load (2 g/kg body wt) and liver glycogen determined after 120 min (H) in rats treated with vehicle (control, n = 5) or MSH5 (5 μmol/kg body wt; n = 3). +P < 0.05 MSH5 vs. vehicle control.

**RESULTS**

Change in responsiveness of hepatocytes to 5-HT during culture. A comparison of the effects of 5-HT on glycogen synthesis in hepatocytes cultured for 2 or 24 h showed that 5-HT (100 μmol/L) stimulated in the 2-h culture model, but inhibited by 60% in 24 h–cultured hepatocytes, whereas insulin stimulated in both models (Fig. 1A). Significant stimulation by 5-HT in 2-h–cultured hepatocytes occurred at concentrations ≥10 mM (Fig. 1B, inset) with maximal stimulation at micromolar concentrations (Fig. 1B), as shown for 5-HT bioactivity in other cell types (28). mRNA levels for 5-HT receptors were determined at 2- and 24-h culture. GAPDH and cyclophilin mRNA levels (normalized to total RNA) were the same at 2 and 24 h (GAPDH, 1.03 ± 0.13; cyclophilin, 1.14 ± 0.11; 24/2 h; n = 10). However, expression of the 5-HT2A receptor declined by 70% at 24 h (0.32 ± 0.05; n = 10; P < 0.00001) whereas expression of 5-HT1B, 5-HT2B, and 5-HT6 receptors increased three- to fivefold at 24 h relative to 2 h (5-HT1B, 4.78 ± 0.71, n = 10, P < 0.0003; 5-HT2B 2.7 ± 0.3, n = 14, P < 0.0003; 5-HT6, 3.37 ± 0.28, n = 6, P < 0.03). Expression of other 5-HT receptors was either unchanged or below detection limits. Because of the changes in 5-HT receptor expression that occur during 24-h culture, in the rest of this study, hepatocytes were used 2-3 h after plating except for Fig. 7, which involved 24-h culture to allow protein expression.

Expression of results.

Results are expressed as means ± SEM, for the Student paired t test for in vitro studies and unpaired t test for in vivo studies.
synthesis in hepatocytes (Fig. 1C). Methyl-5HT was used in the rest of this study to determine the mechanism of the stimulatory pathway without interference from the inhibitory (SB-206553 sensitive) pathway(s).

**Effects on glycolysis.** Insulin caused modest stimulation of glycogen synthesis and glycolysis and elevation in fructose 2,6-P₂, an activator of glycolysis (Fig. 2A–C). Methyl-5HT caused greater stimulation of glycogen synthesis (Fig. 2A) but did not stimulate glycolysis, and it counteracted the elevation of glycolysis and fructose 2,6-P₂ by insulin. It also lowered fructose 2,6-P₂ (Fig. 2C). 5-HT and methyl-5HT caused modest inhibition of glycolysis and this was reversed with the 5-HT1B antagonist (Fig. 2D). This indicates selective stimulation of glycogen synthesis, but not glycolysis, by 5-HT and methyl-5HT.

**Stimulation of glycogen synthesis is mediated by inactivation of phosphorylase.** Candidate mechanisms for stimulation of hepatic glycogen synthesis include activation of glucokinase, inhibition of glucose-6-phosphatase, inactivation of glycogen phosphorylase (dephosphorylation of active GPa to inactive GPb), and activation of glycogen synthase. The latter mechanisms are not mutually exclusive because GPa inhibits activation of glycogen synthase and, accordingly, depletion of GPa enables glycogen synthase activation (33,34). To test for these mechanisms, we compared the effects of methyl-5HT with a glycogen phosphorylase inhibitor (GPI; CP-91149) that converts GPa to GPb (24), and we determined the combined effects on glycogen synthesis of methyl-5HT or the GPI with a glucokinase activator (0.2 mmol/L sorbitol) or a glucose-6-phosphatase.
inhibitor (2 μmol/L S4048) (35). Methyl-5HT depleted GPa and activated glycogen synthase with a similar time course as the GPI (Fig. 3A and B). Stimulation of glycogen synthesis by methyl-5HT was additive with stimulation by sorbitol or S4048 but not by the GPI (Fig. 3C). Likewise stimulation by the GPI was additive with stimulation by sorbitol or S4048, but not methyl-5HT (Fig. 3D). In incubations with the GPI and/or methyl-5HT (but not sorbitol or S4048), GPa activity correlated with glycogen synthesis ($r = -0.99; P < 0.02$) (Fig. 3D vs. F) and glycogen synthase activity ($r = -0.99; P < 0.02$) (Fig. 3E vs. F), supporting a role for GPa depletion in activation of glycogen synthesis and stimulation of glycogen synthesis. The GPI did not inhibit glycolysis (control 176 ± 19, 10 μmol/L CP-91149 176 ± 18 mmol/h/mg; $n = 6$), indicating that inhibition of glycolysis by 5-HT or methyl-5HT (Fig. 2D) is not secondary to stimulation of glycogen synthesis.

**Inactivation of glycogen phosphorylase by a mechanism downstream of cAMP-dependent protein kinase.** Conversion of GPa to Gpb could result from inhibition of phosphorylase kinase (Phk) or activation of phosphorylase phosphatase (Fig. 4A). Phk is activated by protein kinase (PKA) (cAMP-dependent protein kinase) or by calcium via calmodulin (8). To test for mechanisms upstream of PKA, we determined whether methyl-5HT attenuates phosphorylation of GPa by PKA, we determined whether methyl-5HT attenuates phosphorylase kinase (Phk) or activation of phosphorylase phosphatase, hepatocytes were treated with methyl-5HT for 5 min and then incubated with glucagon (1 or 10 nmol/L) for 60 s. Phosphorylation of GPa by methyl-5HT but not insulin or the GPI (Fig. 6A). Two other cdk inhibitors, purvalanol and kenpaullone (36), also attenuated the glycogenic stimulation by methyl-5HT (not shown). Roscovitine counteracted the inactivation of GPa by 5-HT and methyl-5HT but not by resorcinol, an indirect inhibitor of Phk (38) or the GPI (Fig. 6B). The efficacy of roscovitine was dependent on agonist concentration (Fig. 6C and D).

**Adaptive regulation of p35/cdk5 and effects on glycogen synthesis.** Cdk5 is a roscovitine-sensitive, proline-directed kinase that is expressed ubiquitously and has no intrinsic kinase activity in the absence of the coactivator proteins p35 (cdk5R1) or p39 (cdk5R2) (37). We confirmed expression in hepatocytes at the mRNA level of cdk5 and p35, and the p35 regulator cdkal1, but not p39. Expression of p35 mRNA (but not cdkal1) was elevated 2.5-fold by treatment with 25 mmol/L glucose for 4 h (Fig. 7A), as was shown for pancreatic islets (39). When p35 was expressed with an adenoviral vector, p35 immunoreactivity was enhanced during incubation with 25 mmol/L glucose or 10 μmol/L methyl-5HT (Fig. 7B), suggesting stabilization of p35 protein. The phosphorylation of PPI-Thr320, which is a substrate for cdk5 (40), was enhanced by overexpression of p35 or by incubation with methyl-5HT or 25 mmol/L glucose, consistent with activation of cdk5 (Fig. 7C). Overexpression of p35 and cdk5 with adenoviral vectors augmented glycogen synthesis in hepatocytes cultured for 24 h, and this was additive with the stimulation by methyl-5HT (Fig. 7D), supporting a role for cdk5 in control of glycogen synthesis.

**DISCUSSION**

Physiological studies have indicated a role for hepatic nerves in regulating postprandial hepatic glucose disposal by a mechanism that is more rapid than insulin action (3–5,41). 5-HT stimulates hepatic glucose uptake and inhibits endogenous glucose production when infused intraportally but the underlying mechanism is unresolved (17). Because serotonergic nerves are abundant in human liver (15) and several 5-HT1 and 5-HT2 receptors are expressed in liver (19), we used ligands for these receptors to test for novel signaling mechanisms on hepatic glucose metabolism that could be involved in the postprandial state (41). We show that 5-HT stimulates glycogen synthesis in freshly isolated hepatocytes as a result of opposing stimulatory and inhibitory mechanisms mediated by distinct receptors (Fig. 8). The involvement of 5-HT1 and 5-HT2A receptors in the 5-HT stimulatory response is supported by the counteraction by antagonists of 5-HT1 and 5-HT2A receptors and by olanzapine, an antagonist of 5-HT1B and 5-HT2A receptors (31,32), and by the greater efficacy of agonists of 5-HT1...
FIG. 3. Stimulation of glycogen synthesis by methyl-5HT is not additive with the stimulation by a GPI. Time course of GPa depletion (% time zero) and activation of glycogen synthase (% CP-91149 at 60 min) by 100 μmol/L methyl-5HT (M5HT) (A) or 10 μmol/L CP-91149 (GPI) (B); n = 3.

C: Glycogen synthesis (nmol/3 h/mg), combined effects of 100 μmol/L M5HT (black bars) with a glucokinase activator (GKA; 200 μmol/L sorbitol), a glucose-6-phosphatase inhibitor (G6PI; 2 μmol/L S4048), and a GPI (40 μmol/L CP-91149); n = 3. *P < 0.05 effect of M5HT. Combined effects of the GPI (25 μmol/L CP-91149; black bars) with sorbitol (200 μmol/L), S4048 (2 μmol/L), and methyl-5HT (100 μmol/L) on glycogen synthesis (nmol/3 h/mg) (D), GPa activity (E), and glycogen synthase (G. Synthase) activity (F); n = 3. *P < 0.05 vs. control (Con); #P < 0.05 GPI and other additions vs. GPI alone.

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FIG. 4. Methyl-5HT (M5HT) inactivates glycogen phosphorylase by a mechanism downstream of PKA. 

A: Glucagon activates PKA, causing phosphorylation of PFK2 (Ser32) and Phk, which phosphorylates GPb to GPa. Time course of the effect of glucagon (10 nmol/L) without (○) or with (●) 100 μmol/L methyl-5HT on GPa enzyme activity (B), GPa(P) immunoactivity (C), and PFK2-Ser32(P) immunoactivity (D); n = 4. Results expressed as % of glucagon 2 min. *P < 0.05 effect of methyl-5HT. 

E and F: Hepatocytes were incubated for 1 h with the concentrations of cpt-cAMP indicated without or with 100 μmol/L methyl-5HT (M5HT). E: GPa activity at the end of incubation. F: Increment in GPa activity caused by cpt-cAMP above corresponding control; n = 4. *P < 0.05 effect of methyl-5HT. 

G and H: Hepatocytes were incubated with methyl-5HT (100 μmol/L for 5 min) followed by glucagon (1 or 10 nmol/L for 60 s), and phosphorylase phosphatase (PP) activity was determined as described in the RESEARCH DESIGN AND METHODS. G: Representative time course of phosphorylase phosphatase activity determined from the decline in GPa activity (□) and GPa(P) immunoactivity (△) showing immunoblot for GPa(P) and total glycogen phosphorylase (GP). H: Phosphorylase phosphatase activity (% GPa depletion) after 10 min; n = 6. *P < 0.05 effect of methyl-5HT.
and 5-HT2A receptors compared with 5-HT, which interacts with multiple receptors. The involvement of inhibitory pathway(s) is supported by the enhancement of 5-HT stimulation with the 5-HT2BC antagonist, by the switch from stimulation to inhibition by 5-HT after 24-h culture in association with elevated 5-HT2B mRNA expression, and by the greater efficacy of 5-HT1/2A receptor agonists, which, unlike 5-HT, are not affected by the 5-HT2BC antagonist. Methyl-5HT, an agonist of 5-HT1/2A receptors (30), stimulated glycogen synthesis with a similar time course and almost comparable efficacy as pharmacological inhibitors of glycogen phosphorylase and increased hepatic glycogen after oral glucose delivery in vivo with concomitant suppression of blood glucose, consistent with its efficacy in hepatocytes.

In view of the large diversity of 5-HT receptors by way of gene number and splice variants (18) and the formation of 5-HT receptor heterodimers (18), multiple 5-HT receptors as homodimers or heterodimers may be involved in both the glycogenic stimulation and inhibition by 5-HT. The changes in 5-HT receptor expression during culture involving increased expression of 5-HT2B and decreased expression of 5-HT2A receptors, as shown by both changes in mRNA levels and also altered responses to the respective agonists and antagonists, are not a feature shared by all G protein–coupled receptors, because glucagon receptors do not change (42). It may be a property of receptors involved in hepatocyte proliferation because 5-HT2A/2B receptors (19), similar to adrenergic receptors, are implicated in liver regeneration (43). Alternatively, it could be a property of neurotransmitter receptors because adrenergic receptors mediate the effects of sympathetic nerves on hepatocytes (8,9). Whether changes in the ratio of stimulatory/inhibitory 5-HT receptors in hepatocytes have a physiological role remains to be determined. Expression of multiple 5-HT receptors within a cell (18) with opposing metabolic effects enables both diversity of response to the ligand and sensitivity to a mechanism modulating receptor expression. The opposite metabolic effects of 5-HT mediated through different receptors could explain why antidepressants targeting serotonin function increase the risk for both hypoglycemia and hyperglycemia (22).

Two major sites of control of hepatic glycogen synthesis are glucokinase and glycogen phosphorylase (33,34). These enzymes exert an unusually high control strength on glycogenic flux. A large response is necessary to mediate the metabolic switch from net glycogen degradation to synthesis in the fasted-to-fed transition. Whereas glucokinase activation increases flux through glycogen synthesis and glycolysis, inactivation of glycogen phosphorylase selectively promotes glycogen storage. In this study, both 5-HT and methyl-5HT caused selective stimulation of glycogen synthesis but not glycolysis. This contrasts with the stimulation of glycolysis by insulin or elevated glucose. The actions of elevated glucose and insulin on the liver can be rationalized as mechanisms to restore blood glucose homeostasis in hyperglycemia by enhancing hepatic glucose clearance. In contrast, the action of 5-HT (through 5-HT1/2A receptors) seems geared towards selective replenishment of glycogen stores and, moreover, is characterized by rapid inactivation of glycogen phosphorylase. This implicates

FIG. 5. 5-HT, but not acetylcholine, counteracts phosphorylase activation by norepinephrine. A: GPa activity in hepatocytes incubated for 5 min with or without 30 μmol/L methyl-5HT (M5HT, ■) before addition of epinephrine (EP, 10 μmol/L), norepinephrine (NE, 10 μmol/L), or glucagon (GLN, 10 μmol/L) for 60 s; n = 4. *P < 0.01 effect of M5HT. B: GPa activity in hepatocytes incubated for 5 min with 100 μmol/L 5-HT or 100 μmol/L acetylcholine (ACC) followed by the addition of norepinephrine (5 μmol/L, ■) for 60 s; n = 4. **P < 0.01 relative to norepinephrine alone. C: Hepatocytes were incubated with antagonists (as in Fig. 1D) for 10 min followed by 100 μmol/L 5-HT for 5 min followed by norepinephrine (5 μmol/L) for 60 s; n = 4. *P < 0.05 relative to norepinephrine alone.
potential involvement in the portal signal, which is associated with elevated glycogen but not lactate production (3) and represents a more rapid response than insulin action with maximal effect within 15 min (41). The function of the inhibitory 5-HT receptor(s) could be a safety mechanism to prevent excessive glycogen storage or an amplification mechanism to enable the rapid adaptive changes in response to 5-HT.

Stimulation of glycogen synthesis by 5-HT receptor agonists can be fully accounted for by inactivation of glycogen phosphorylase. At least two mechanisms can mediate this inactivation of glycogen phosphorylase: activation of phosphorylase phosphatase and counteraction of Phk activation by norepinephrine (Fig. 8). The latter might involve dephosphorylation of Phk by a protein phosphatase, modulation of calcium homeostasis, or possibly also interaction with adrenergic receptor signaling. A role for activation of phosphorylase phosphatase was supported by the dephosphorylation of GPa but not PFK2 in the presence of glucagon and by elevated phosphorylase phosphatase activity. This comprises the catalytic unit of PP1 in association with one or more regulatory units (44). Activation of phosphorylase phosphatase could result from increased binding of PP1 to a glycogen-targeting protein or altered interaction with inhibitory units (44). A role for a cdk5-dependent mechanism in 5-HT regulation is supported by

![Figure 6](https://example.com/figure6.png)

**FIG. 6.** Roscovitine counteracts the action of 5-HT and methyl-5HT. *A:* Glycogen synthesis (nmol/3 h/mg; n = 5) in hepatocytes incubated with 10 nmol/L insulin (Ins), 10 μmol/L methyl-5HT (M5HT), or 10 μmol/L CP-91149 (GPI) without or with 50 μmol/L roscovitine. *P < 0.006 effect of roscovitine. B: GPa activity determined after 1 h incubation with 100 μmol/L 5-HT, 10 μmol/L M5HT, 150 μmol/L resorcinol (Res), or 10 μmol/L CP-91149 (GPI) without or with 50 μmol/L roscovitine; n = 3. **P < 0.003 effect of roscovitine; #P < 0.05 relative to untreated control (Con).

Glycogen synthesis (nmol/3 h/mg; n = 4) (C) and GPa activity (mU/mg; n = 3) (D) at varying M5HT concentration ± 50 μmol/L roscovitine. *P < 0.05; **P < 0.02 effect of roscovitine.
counterregulatory effects of the cdk inhibitors on phosphorylase inactivation and glycogen synthesis stimulation by 5-HT and methyl-5HT but not insulin; 2) augmented glycogen synthesis by overexpression of cdk5 and its coactivator p35; 3) adaptive regulation of p35 by methyl-5HT; and 4) increased phosphorylation of the cdk5 substrate PP1-Thr320 by methyl-5HT (40). Cdk5 was first identified in brain and later shown to be expressed ubiquitously (37). Its activity is dependent on activator proteins (p35/cdk5R1 and p39/cdk5R2) with a short half-life (37). A role for cdk5 in blood glucose homeostasis is suggested by the association between a common polymorphism in a cdk5 regulatory unit (CDKAL1) and type 2 diabetes (45). The yeast homolog of cdk5 (Pho85) is involved in regulation of phosphorylase phosphatase (25), but a role for cdk5 in regulation of mammalian glycogen metabolism has not been reported.
and that the stimulatory receptors lower blood glucose and hepatic glycogen metabolism mediated by distinct receptors.

A potential tool to test for involvement of 5-HT in hepatic genic stimulation by 5-HT suggests that this drug may be determined. The counteraction by olanzapine of the glyco-

intraportal glucose in animal models (7,50). Whether 5-HT

tylcholine, inhibited phosphorylase activation by norepi-

nepinephrine. (ARs) activates Phk, and 5-HT and methyl-5HT, acting through 5-HT1 receptors, counterregulate the activation of glycogen phosphorylase by norepinephrine.

FIG. 8. Mechanism of action of 5-HT on hepatic glycogen metabolism. 5-HT stimulates glycogen synthesis through 5-HT1 and 5-HT2A receptors via a mechanism involving activation of cdk5/p35 and activation of phosphorylase phosphatase (Ph-P). resulting in dephosphorylation of GPa, the active form of glycogen phosphorylase, which is a potent inhibitor of glycogen synthase phosphatase, comprising PP1 and the glycogen-targeting protein, Gl (GL). This catalyzes the dephosphorylation/activation of glycogen synthase (conversion of GSB to GSa). Interaction of 5-HT with 5-HT1B/C receptors has a counterregulatory effect and opposes the stimulation of glycogen synthesis. Methyl-5HT (MHT) binds to the 5-HT1 and 5-HT2A receptors and induces only the stimulatory mechanism. Norepinephrine (NE) acting through adrenergic receptors (ARs) activates Phk, and 5-HT and methyl-5HT, acting through 5-HT1 receptors, counterregulate the activation of glycogen phosphorylase by norepinephrine.

Several inhibitor proteins and also some glycogen-targeting proteins of PP1 (44) have a proline-directed cdk consensus motif (37) and/or have been shown to be phosphorylated by cdk5 (46,47). These PP1-binding proteins could be involved in mediating the effects of 5-HT on phosphorylase or Phk. Cdk5 is involved in the signaling mechanisms of various neurotransmitters, including dopamine and the metabotropic glutamate receptors (48,49).

In addition to transcriptional regulation and turnover of p35 protein, covalent modification of both cdk5 and p35 by protein kinases and calcineurin is involved in neurotransmitter signaling (37). The mechanisms linking 5-HT receptors with cdk5 activity in hepatocytes remain to be determined.

A key finding from this study is that 5-HT, but not ace-
typhilcholine, inhibited phosphorylase activation by norepi-

nephrine (Fig. 8). Inhibition of sympathetic transmission is implicated in the stimulation of hepatic glucose uptake by intraportal glucose in animal models (7,50). Whether 5-HT has a physiological role in stimulating hepatic glucose uptake during oral glucose ingestion in humans remains to be determined. The counteraction by olanzapine of the glyco-

genic stimulation by 5-HT suggests that this drug may be a potential tool to test for involvement of 5-HT in hepatic glucose disposal.

In summary, we show that serotonin has dual effects on hepatic glycogen metabolism mediated by distinct receptors and that the stimulatory receptors lower blood glucose and stimulate hepatic glycogen synthesis rapidly and with high efficacy. The mechanism involves inactivation of glycogen phosphorylase and inhibition of the glygenolytic effect of norepinephrine. This implicates potential involvement of serotonin in the metabolic transition from glycogen depletion to storage after a meal. It provides insight into why drugs targeting serotonin function can cause hyperglycemia or hypoglycemia (22).

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