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

Susan J. Tudhope,[1] Chung-Chi Wang,[1] John L. Petrie,[1] Lloyd Potts,[1] Fiona Malcomson,[1] Julius Kieswich,[2] Muhammad M. Yaqoob,[2] Catherine Arden,[1] Laura J. Hampson,[1] and Loranne Agius[1]

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-HT1/2A and 5-HT2B receptors, respectively. Agonists of 5-HT1/2A receptors lowered blood glucose and increased hepatic glycogen after oral glucose loading and also stimulated glycogen synthesis in freshly isolated hepatocytes with greater efficacy than 5-HT. This effect was blocked by olanzapine, an antagonist of 5-HT1/2A 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 glycolysis and was counteracted by cyclin-dependent kinase (cdk) 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. ***Diabetes*** **61:49–60, 2012**

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 hypoglucagonemia (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 serotonergic 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 nervous systems and a hormone produced by the gut and transported in platelets (16). It acts on target cells through 5-HT receptors encoded by 14 genes and involving numerous splice variants (18). Platelet-derived 5-HT is implicated in liver regeneration, which is associated with adaptive changes in expression of multiple 5-HT receptors in liver (19). The role of specific hepatic 5-HT receptors in mediating the effects of 5-HT on glucose metabolism remains unexplored. We previously reported that in hepatocytes cultured for 24 h, 5-HT inhibits glycogen synthesis at micromolar concentrations but causes modest stimulation at nanomolar concentrations (20). Subsequent studies showed that the expression of 5-HT receptors in hepatocytes changes during a 24-h culture. In this study, we used freshly isolated hepatocytes to identify specific 5-HT receptors and signaling pathways involved in regulation of hepatic glucose metabolism. We show that 5-HT can induce either stimulation or inhibition of glycogen synthesis through distinct receptors. The opposing metabolic effects of distinct 5-HT receptors could explain why drugs targeting 5-HT function can cause either diabetes or hypoglycemia (21,22).

RESEARCH DESIGN AND METHODS

Reagents. 5-HT, α-methyl-5HT, and (+/−)-2,5-dimethoxy-4-iodoamphetamine were from Sigma-Aldrich (St. Louis, MO). Other agonists and antagonists were from Tocris Bioscience (Bristol, U.K.). CP-91149 was a gift from Pfizer Global Research and Development (Groton, CT) and S4048 was from sanofi-aventis Deutschland GmbH (Frankfurt am Main, Germany).

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. 1*A* 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)α adenoviral vectors (Vector Biolabs, Philadelphia, PA), the serum-containing medium was replaced after 2 h with

From the [1]Institute of Cellular Medicine, The Medical School, Newcastle University, Newcastle upon Tyne, U.K.; and the [2]Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Queen Mary University of London, London, U.K.
Corresponding author: Loranne Agius, loranne.agius@ncl.ac.uk.
Received 22 June 2011 and accepted 30 September 2011.
DOI: 10.2337/db11-0870
S.J.T. and C.-C.W. contributed equally to this study.
© 2012 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

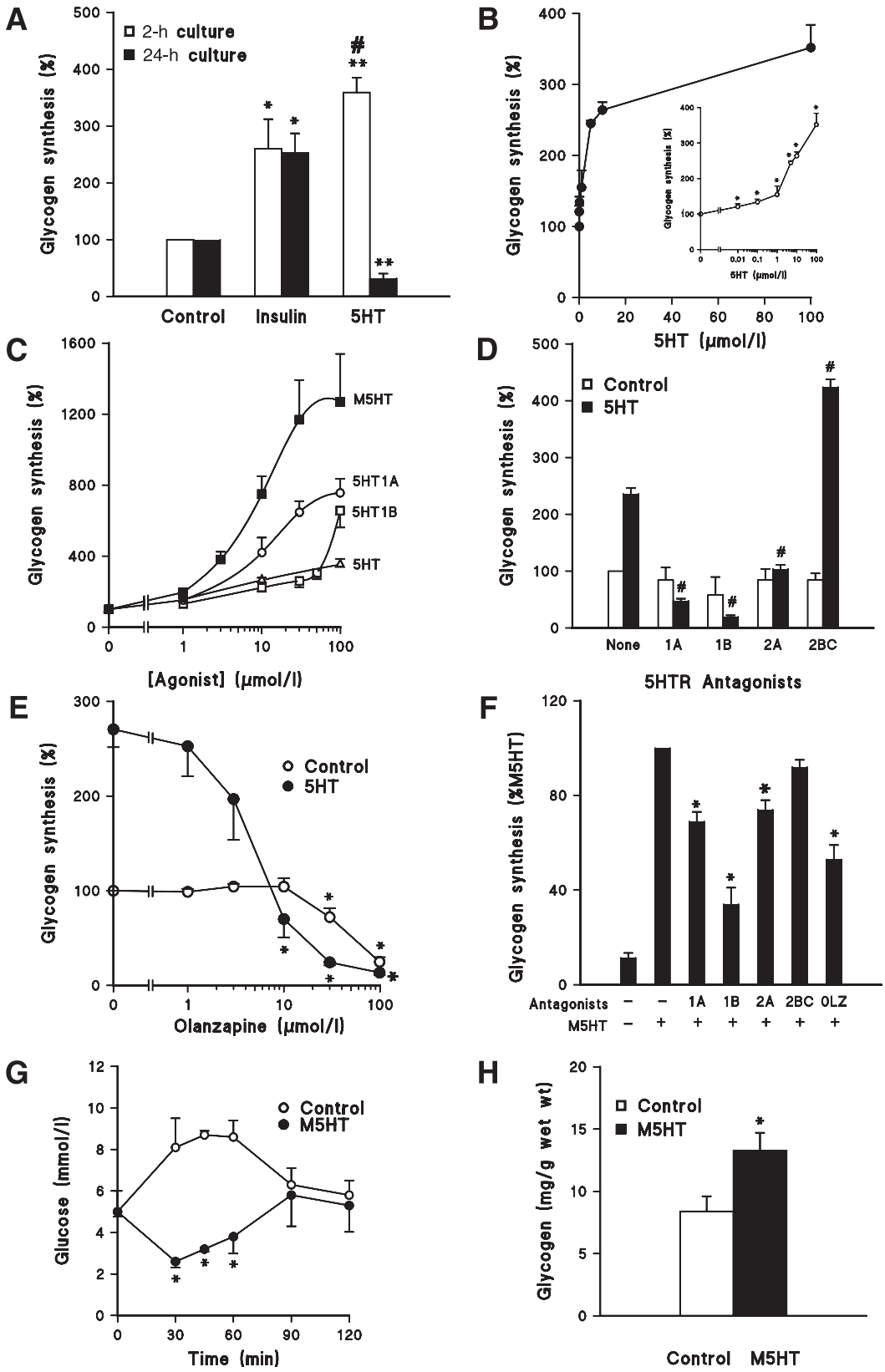

FIG. 1. Effects of 5-HT receptor agonists on glycogen synthesis in vitro or in vivo. *A*: Glycogen synthesis determined during a 3-h incubation without or with 50 nmol/L insulin or 100 μmol/L 5-HT in hepatocytes precultured for 2 or 24 h, expressed as percentage of respective control. Basal rates of 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 (M5HT), 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 nmol/L dexamethasone and 5 mmol/L 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 mmol/L glucose and [U-^{14}C]glucose (2 μCi/mL) (20), and glycolysis from 1- or 3-h incubations in medium containing 15 mmol/L glucose and [3-^{3}H]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 mmol/L KF (potassium fluoride), 25 mmol/L HEPES, 0.5 mmol/L EDTA, 1 mmol/L 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 mmol/L 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 mU is the amount of enzyme converting 1 nmol substrate per minute. Fructose 2,6-bisphosphate (fructose 2,6-P_2) was determined as previously described (26).

Immunoblotting. The cells were extracted in gel loading buffer (30 mmol/L 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 dried 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 phosphopeptides: GPa-Ser14 (27), phosphofructokinase-2 (PFK2)/fructose bisphosphatase-2-Ser32 (26), and PP1α-Thr320 or PP1α (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 was performed in a 20-μL volume containing 100 ng of reverse-transcribed RNA in a Roche Light Cycler-480 using TaqMan gene expression assays (Applied Biosystems) for 5-HT1A (Rn00561409_s1), 5-HT1B (Rn01637747_s1), 5-HT1F (Rn00573147_s1), 5-HT2A (Rn00573147_s1), 5-HT2B (Rn00573147_s1), and 5-HT2C (Rn00562748_m1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Rn99999916_s1) and cyclophilin (Rn00690933_m1) were used as internal reference for 5-HT1 (nonexon spanning) and 5-HT2 receptors, respectively. SYBR green RT-PCR (Roche Applied Science) was used for 5-HT1A (F5′-GGGCAGTGAG GCAGGGTGAC-3′, R5′-GGCGGGCAAGGAGTTAGAT-3′); 5-HT1B (F5′-CAC GGTGGGGGCTTTCTATTTAC-3′, R5′-TGCCGGTCTTGTTGTGTCT-3′); 5-HT1F (5′-AGTCGAGATGCCAGGAAGAGGA-3′, R5′-GAGGAGGCACGGAGATGAACAC-3′); 5-HT2B (F5′-TTTGGGTCACTGCTGCTTTCTTT-3′, R5′-AGTCCACCGTGTAGGG TGA-3′); 5-HT2C (F5′-CACACCGAGGAGGAACTGGCTAATA-3′, R5′-TCTGGGAC GCTTTTCTTTCTTCTTT-3′); 5-HT6 (F5′-GGAGCTCTCAGCCCACTCT-3′, R5′-TCA GCACACTCTCAGGAACC-3′); cdk5 (F5′-CATCCTTGGTGAATGTCGTG, R5′-CAG AGAGTCTACGGGGGACA); p35/cdk5R1 (F5′-TCATGAGCTCCAAGATGCTG, R5′-GCCAGGATACCAGCTACAGG); and cyclophilin (F5′-ATGGCACTGGTGGCAAGTCC, R5′-TTGCCATTCCTGGACCCAAA).

Expression of results. Results are expressed as means ± SEM, for the number (n) of hepatocyte preparations indicated. Statistical analysis was by the Student paired t test for in vitro studies and unpaired t test for in vivo studies.

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. 1*A*). Significant stimulation by 5-HT in 2 h–cultured hepatocytes occurred at concentrations ≥10 nmol/L (Fig. 1*B*, inset) with maximal stimulation at micromolar concentrations (Fig. 1*B*), 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; $P < 0.000001$) 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 on glycogen synthesis. We used agonists (Fig. 1*C*) and antagonists (Fig. 1*D*) 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. 1*C*). However, agonists of 5-HT1D (L-694247 and GR-46611) and 5-HT2A/2C (*R*[-]-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 counteracted with antagonists of 5-HT1A, 5-HT1B, and 5-HT2A receptors, but it was significantly enhanced with SB-206553, an antagonist of 5-HT2B/C receptors (Fig. 1*D*), indicating a 5-HT–mediated inhibitory mechanism that is sensitive to SB-206553. The stimulation by 5-HT was inhibited with olanzapine (Fig. 1*E*), 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. 1*F*), but unlike 5-HT stimulation (Fig. 1*D*), it was not further enhanced by SB-206553 (Fig. 1*F*), 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. 1*G*) and enhanced glycogen storage (Fig. 1*H*), 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 M5HT (5 μmol/kg body wt; n = 3). *$P < 0.05$ M5HT vs. vehicle control.

synthesis in hepatocytes (Fig. 1*C*). 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-P_2, an activator of glycolysis (Fig. 2*A*–*C*). Methyl-5HT caused greater stimulation of glycogen synthesis (Fig. 2*A*) but did not stimulate glycolysis, and it counteracted the elevation of glycolysis and fructose 2,6-P_2 by insulin. It also lowered fructose 2,6-P_2 (Fig. 2*C*). 5-HT and methyl-5HT caused modest inhibition of glycolysis and this was reversed with the 5-HT1B antagonist (Fig. 2*D*). 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

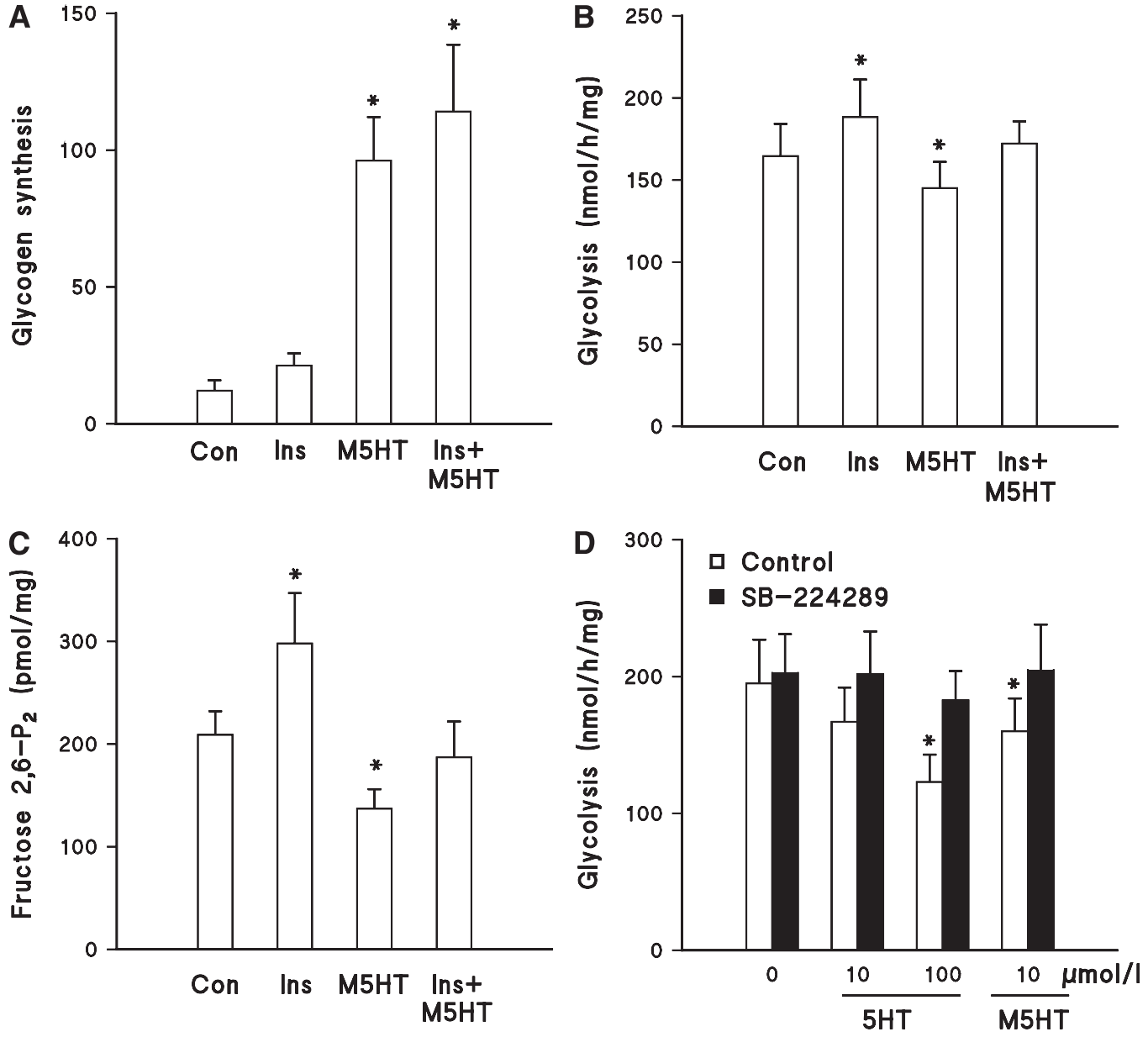

FIG. 2. Comparison of methyl-5HT (M5HT) and insulin (Ins). *A*: Glycogen synthesis (nmol/3 h/mg; $n = 3$) in hepatocytes incubated with 10 nmol/L insulin and/or 30 µmol/L methyl-5HT. *B* and *C*: Glycolysis ($n = 7$) and cellular fructose 2,6-P_2 ($n = 7$) were determined after a 1-h incubation with additions as in *A*. *$P < 0.05$ relative to control (Con). *D*: Glycolysis (nmol/h/mg; $n = 3$) in incubations with 5-HT or methyl-5HT (micromoles per liter) without or with 10 µmol/L SB-224289 (5-HT1B antagonist). *$P < 0.05$ effect of agonist.

inhibitor (2 μmol/L S4048) (35). Methyl-5HT depleted GPa and activated glycogen synthase with a similar time course as the GPI (Fig. 3*A* and *B*). Stimulation of glycogen synthesis by methyl-5HT was additive with stimulation by sorbitol or S4048 but not by the GPI (Fig. 3*C*). Likewise stimulation by the GPI was additive with stimulation by sorbitol or S4048 but not methyl-5HT (Fig. 3*D*). 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. 3*D* vs. *F*) and glycogen synthase activity ($r = -0.99$; $P < 0.02$) (Fig. 3*E* vs. *F*), supporting a role for GPa depletion in activation of glycogen synthase and stimulation of glycogen synthesis. The GPI did not inhibit glycolysis (control 176 ± 19, 10 μmol/L; CP-91149 176 ± 18 nmol/h/mg; $n = 6$), indicating that inhibition of glycolysis by 5-HT or methyl-5HT (Fig. 2*D*) 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. 4*A*). 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 the effect of glucagon on conversion of GPb to GPa and phosphorylation of the PKA substrate PFK2/fructose bisphosphatase-2 on Ser32 (26). Glucagon caused rapid elevation in GPa, as shown by enzyme activity (Fig. 4*B*) and immunoblotting for the NH_2-terminal phosphoserine (Fig. 4*C*), and it increased phosphorylation of PFK2-Ser32 (Fig. 4*D*). Methyl-5HT did not prevent the rapid phosphorylation of GPb or PFK2-Ser32 but it caused dephosphorylation of GPa (Fig. 4*C*) but not PFK2-Ser32(P) after 15–60 min (Fig. 4*D*). As GPa and PFK2-Ser32(P) are dephosphorylated by PP1 and PP2A, respectively (Fig. 4*A*), the dephosphorylation of GPa suggests a site of action downstream of PKA, possibly by activation of PP1. Methyl-5HT also depleted GPa when hepatocytes were incubated for 1 h with cpt-cAMP, a nonmetabolizable cAMP analog (Fig. 4*E*), and attenuated the increment in GPa by 10–50 μmol/L but not by 200 μmol/L cpt-cAMP (Fig. 4*F*), indicating counterregulation of GPb/GPa interconversion by methyl-5HT and cpt-cAMP.

Activation of phosphorylase phosphatase. To test whether methyl-5HT activates phosphorylase phosphatase, hepatocytes were treated with methyl-5HT for 5 min and then incubated with glucagon (1 or 10 nmol/L) for 60 s. Phosphorylase phosphatase activity was determined from a 20-min time course of dephosphorylation of endogenous GPa. Depletion of GPa determined enzymatically was associated with dephosphorylation determined by immunoblotting (Fig. 4*G*). Phosphorylase phosphatase activity was higher in cells treated with methyl-5HT, and this effect was greater at the lower glucagon concentration (Fig. 4*H*), similar to the attenuation by methyl-5HT at lower concentrations of cpt-cAMP (Fig. 4*E* and *F*).

Counteraction of norepinephrine activation of phosphorylase. Other mechanisms that could affect interconversion of GPb and GPa include regulation of Phk through changes in calcium (7–9). To test for this possibility, we determined the effects of methyl-5HT on the activation of phosphorylase by norepinephrine, which is calcium mediated (10–12). Hepatocytes were pretreated with methyl-5HT (5 min) and then challenged for 60 s with norepinephrine, epinephrine, or glucagon. Methyl-5HT counteracted the activation of phosphorylase (GPb to GPa conversion) by norepinephrine (Fig. 5*A*). Comparison of the effect of 5-HT and acetylcholine on the norepinephrine stimulation showed counteraction by 5-HT but not acetylcholine (Fig. 5*B*). This effect of 5-HT was prevented with the 5-HT1B antagonist (SB-224289) but was unaffected by antagonists of 5-HT2A and 5-HT2B/C receptors (Fig. 5*C*).

Roscovitine counteracts the glycogenic stimulation. To identify candidate signaling pathway(s) involved in the glycogenic stimulation by methyl-5HT, we tested several inhibitors targeting kinase cascades or phospholipid signaling (L.H., C.-C.W., and L.A., unpublished data). From the compounds tested, we identified the cdk inhibitor roscovitine (36,37) as an inhibitor of the glycogenic stimulation by methyl-5HT but not insulin or the GPI (Fig. 6*A*). Two other cdk inhibitors, purvanalol 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. 6*B*). The efficacy of roscovitine was dependent on agonist concentration (Fig. 6*C* 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. 7*A*), as was shown for pancreatic islets (39). When p35 was expressed with an adenoviral vector, p35 immunoactivity was enhanced during incubation with 25 mmol/L glucose or 10 μmol/L methyl-5HT (Fig. 7*B*), suggesting stabilization of p35 protein. The phosphorylation of PP1-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. 7*C*). 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. 7*D*), 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

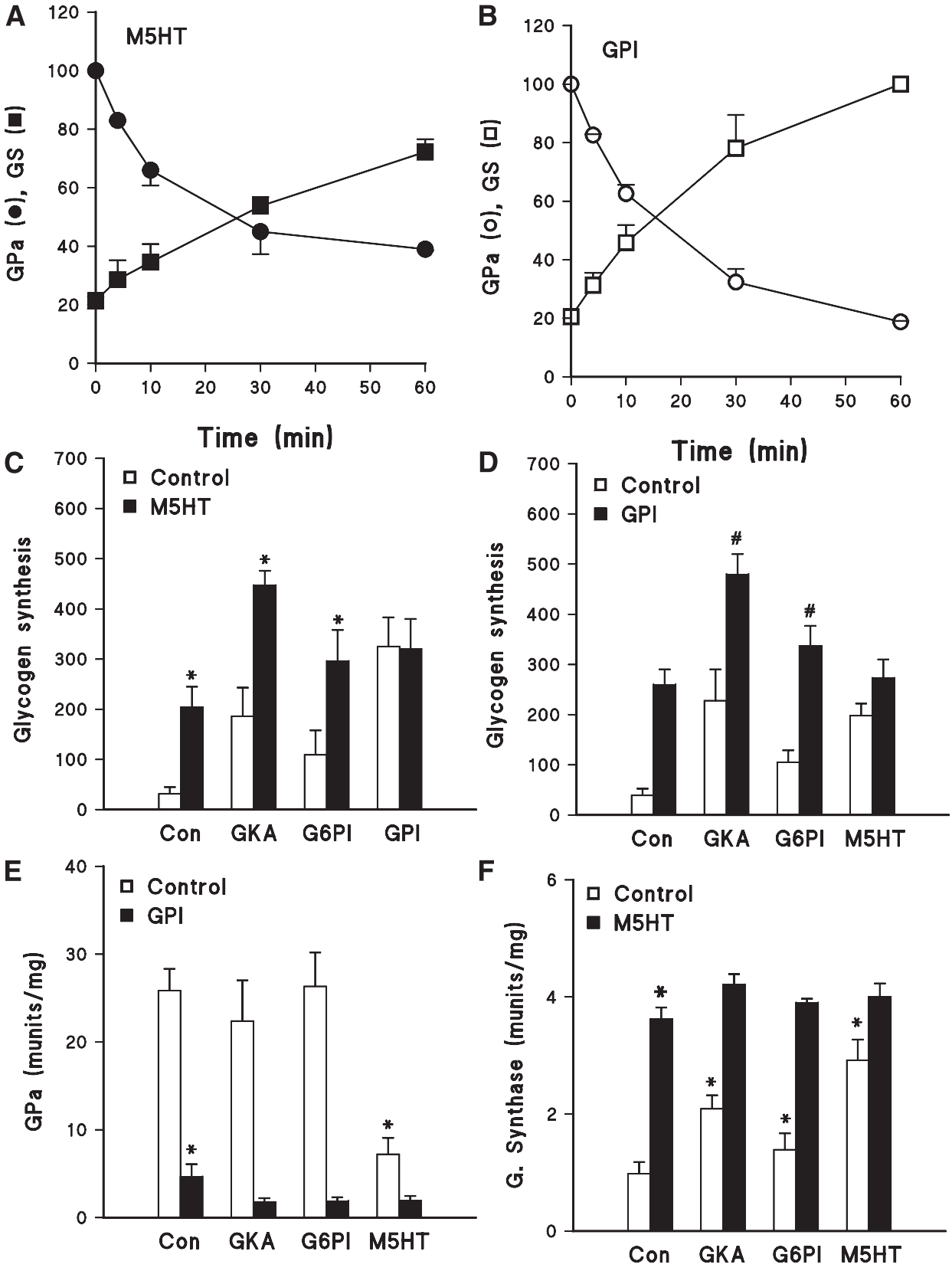

FIG. 3. Stimulation of glycogen synthesis by methyl-5HT is not additive with the stimulation by a GPI. Time course of GPa depletion (% time zero) and activation of glycogen synthase (% CP-91149 at 60 min) by 100 μmol/L methyl-5HT (M5HT) (*A*) or 10 μmol/L CP-91149 (GPI) (*B*); *n* = 3. *C*: Glycogen 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.

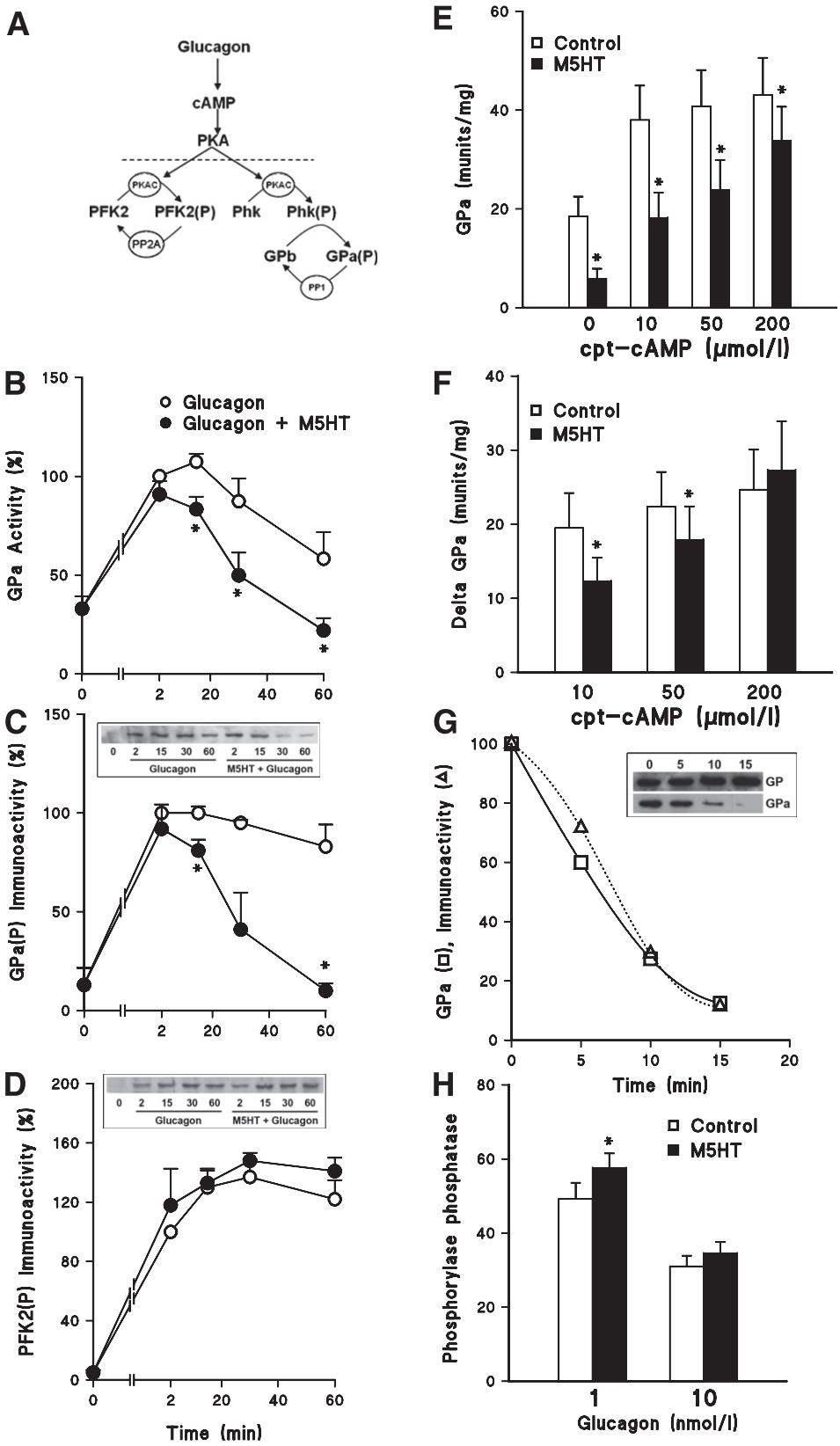

FIG. 4. Methyl-5HT (M5HT) inactivates glycogen phosphorylase by a mechanism downstream of PKA. *A*: Glucagon activates PKA, causing phosphorylation of PFK2 (Ser32) and Phk, which phosphorylates GPb to GPa. Time course of the effect of glucagon (10 nmol/L) without (○) or with (●) 100 μmol/L methyl-5HT 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.

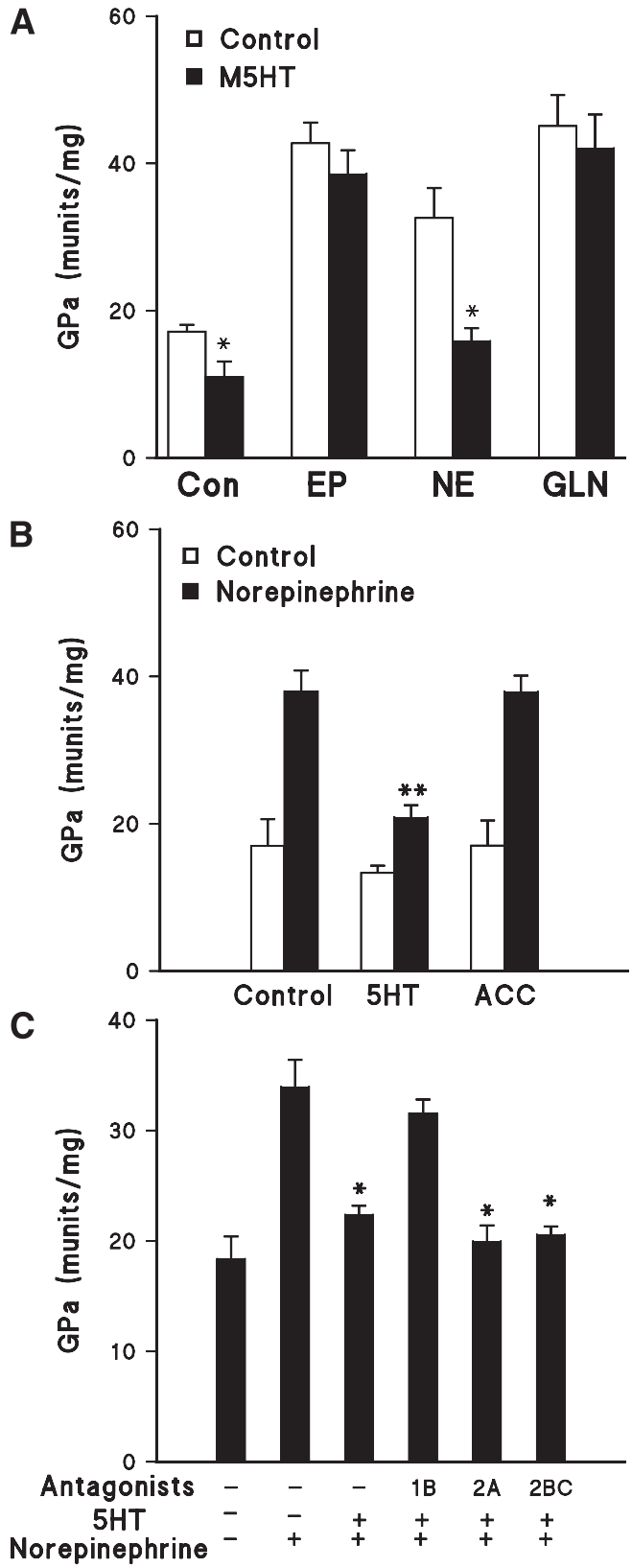

FIG. 5. 5-HT, but not acetylcholine, counteracts phosphorylase activation by norepinephrine. *A*: GPa activity in hepatocytes incubated for 5 min without or with 30 μmol/L methyl-5HT (M5HT, ■) before addition of epinephrine (EP, 10 μmol/L), norepinephrine (NE, 10 μmol/L), or glucagon (GLN, 10 nmol/L) 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. 1*D*) 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.

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), like 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 toward selective replenishment of glycogen stores and, moreover, is characterized by rapid inactivation of glycogen phosphorylase. This implicates

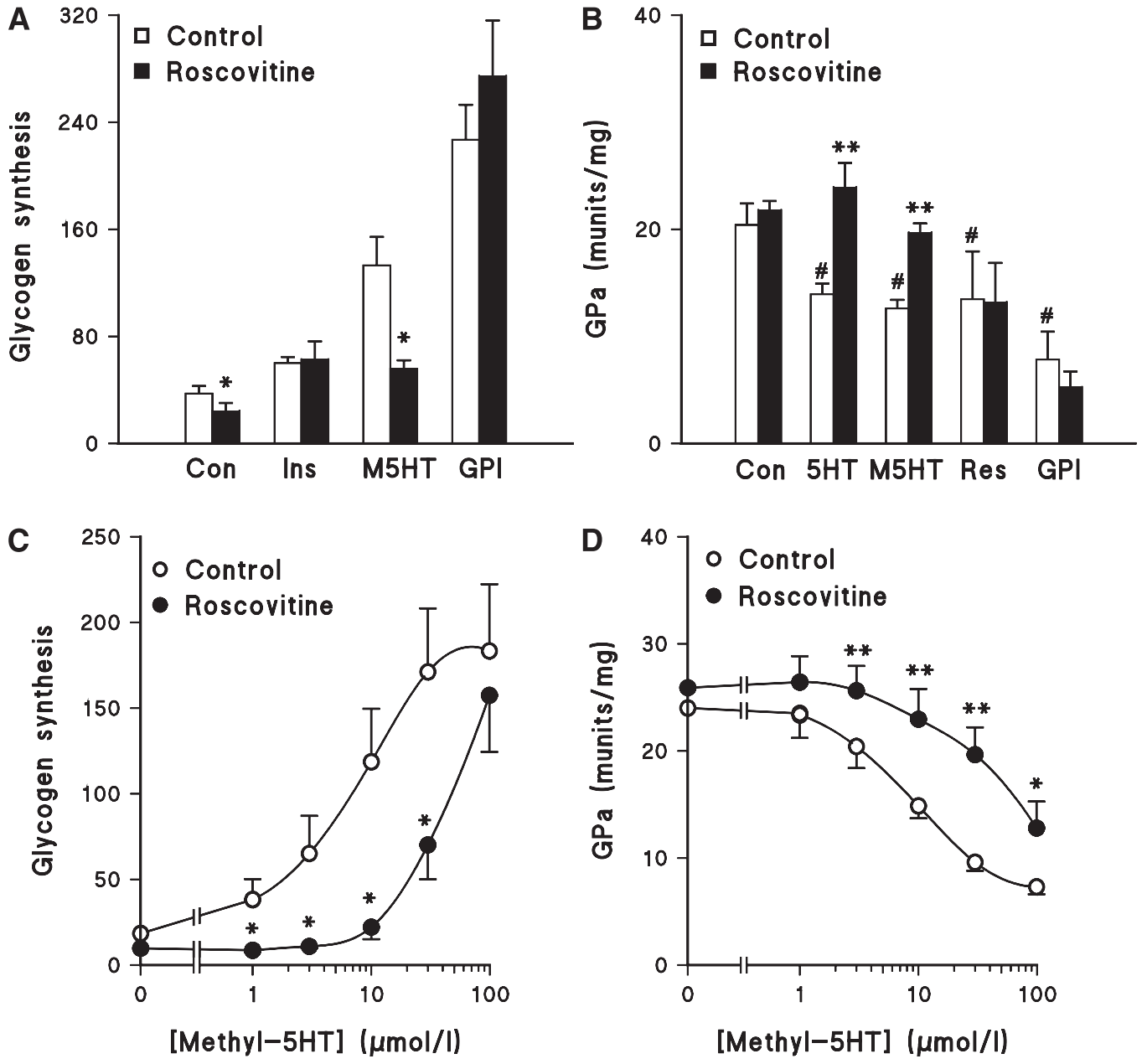

FIG. 6. Roscovitine counteracts the action of 5-HT and methyl-5HT. *A*: Glycogen synthesis (nmol/3 h/mg; $n = 5$) in hepatocytes incubated with 10 nmol/L insulin (Ins), 10 μmol/L methyl-5HT (M5HT), or 10 μmol/L CP-91149 (GPI) without or with 50 μmol/L roscovitine. *$P < 0.006$ effect of roscovitine. *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.

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

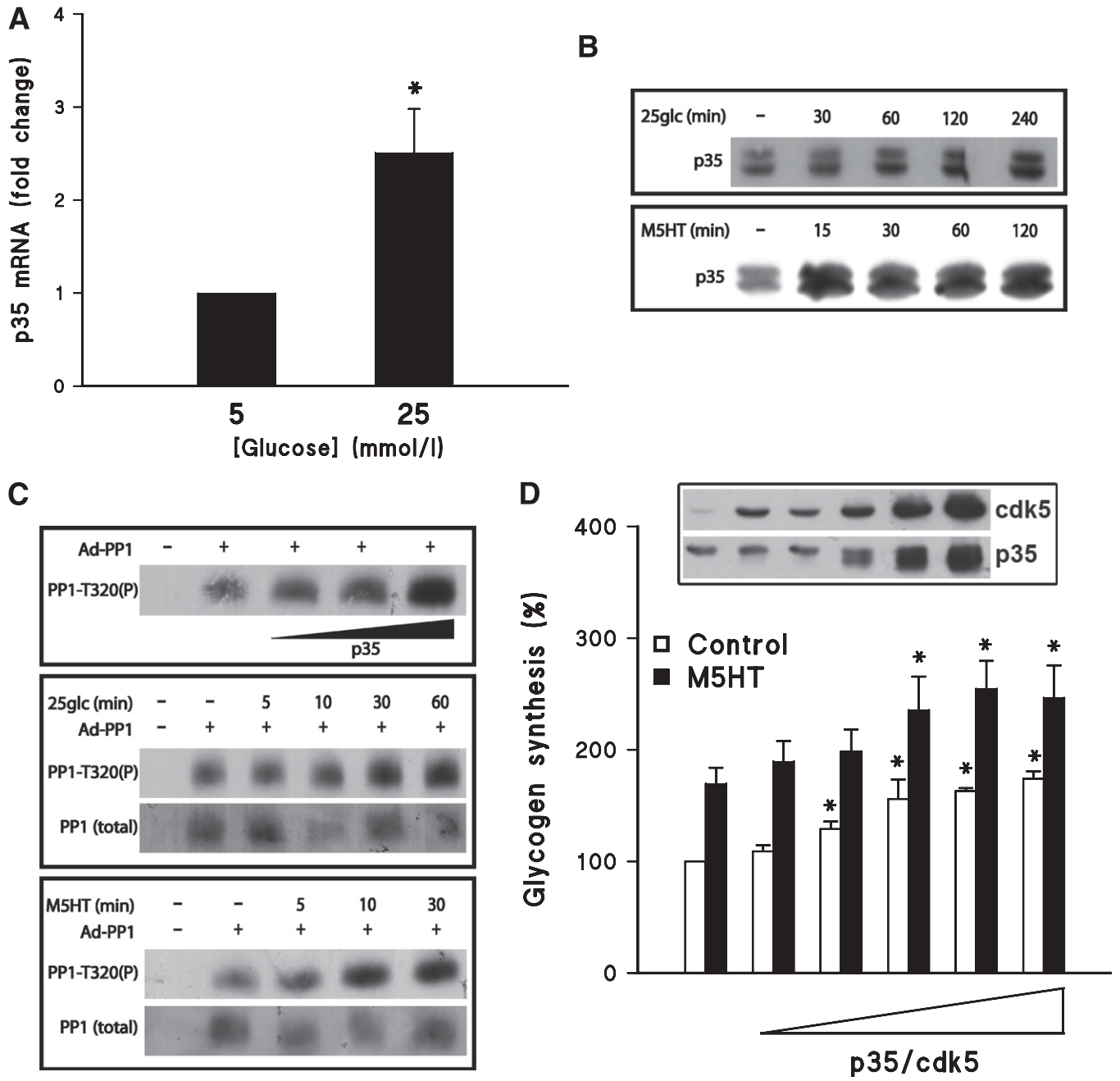

FIG. 7. Involvement of p35/cdk5 in regulation of glycogen synthesis. *A*: Effects of glucose on p35 mRNA. Hepatocytes were precultured for 24 h and then incubated for 4 h with 5 or 25 mmol/L glucose, and p35 mRNA levels at 25 mmol/L glucose are expressed as a ratio to 5 mmol/L glucose (n = 15; *P < 0.01). *B*: Effects of glucose and methyl-5HT (M5HT) on p35 protein. Hepatocytes were treated with adenoviral vector for expression of p35 and cultured for 24 h. They were then incubated for the time intervals indicated with 25 mmol/L glucose (25glc) or 10 μmol/L methyl-5HT, and p35 protein was determined by immunoblotting, representative of two (glucose) and three (M5HT) experiments. *C*: Immunoactivity to PP1-Thr320(P) (PPI-T320(P)) in cells overexpressing PP1 showing effects of p35 overexpression (*top*) and time course of the effects of 25 mmol/L glucose (*middle*) or 10 μmol/L M5HT (*bottom*) on phosphorylation of PP1-Thr320, representative of two or three experiments. Ad, adenoviral. *D*: Effect of titrated overexpression of cdk5 and p35 on glycogen synthesis. Hepatocytes were untreated or treated with five adenoviral titres for expression of cdk5 and p35 and cultured for 24 h. Representative immunoblot shows relative protein expression. They were incubated for 3 h in medium containing 15 mmol/L glucose without or with 10 μmol/L methyl-5HT for determination of glycogen synthesis (expressed as % untreated control; n = 5). *P < 0.05 vs. untreated control.

1) 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.

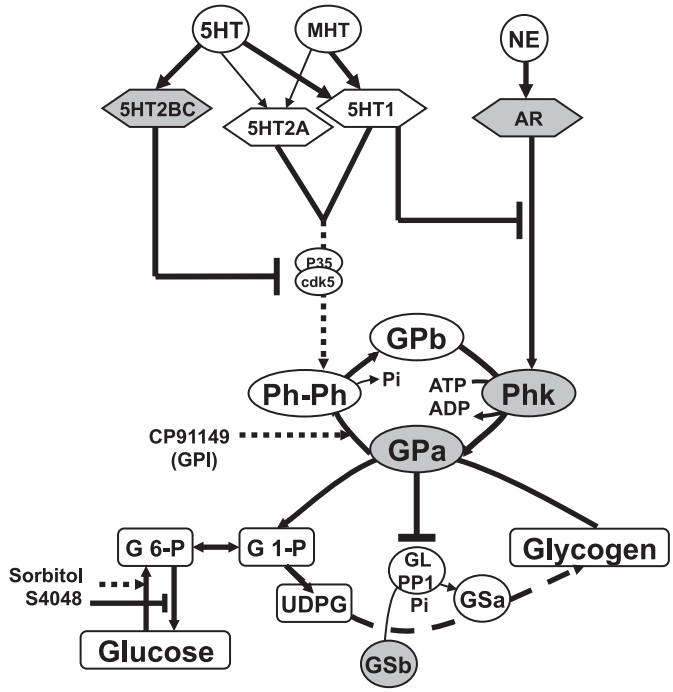

FIG. 8. Mechanism of action of 5-HT on hepatic glycogen metabolism. 5-HT stimulates glycogen synthesis through 5-HT1 and 5-HT2A receptors via a mechanism involving activation of cdk5/p35 and activation of phosphorylase phosphatase (Ph-Ph), resulting in dephosphorylation of GPa, the active form of glycogen phosphorylase, which is a potent inhibitor of glycogen synthase phosphatase, comprising PP1 and the glycogen-targeting protein, G_L (GL). This catalyzes the dephosphorylation/activation of glycogen synthase (conversion of GSb to GSa). Interaction of 5-HT with 5-HT2B/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 acetylcholine, inhibited phosphorylase activation by norepinephrine (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 glycogenic 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 glycogenolytic 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).

ACKNOWLEDGMENTS

This work was funded by Diabetes UK (07/0003489 and 07/003488). J.L.P. was funded by a PhD studentship (07/003559).

No potential conflicts of interest relevant to this article were reported.

S.J.T., C.-C.W., and J.L.P. researched data, contributed to discussion, and reviewed the manuscript. L.P. and F.M. researched data and contributed to discussion. J.K. and M.M.Y. researched data, contributed to discussion, and reviewed the manuscript. C.A. contributed to discussion and reviewed the manuscript. L.J.H. researched data, contributed to discussion, and reviewed the manuscript. L.A. directed the study and wrote the manuscript.

The authors thank Dr. Dieter Schmoll (sanofi-aventis Deutschland GmbH) for S4048 and reagents, Dr. Judy Treadway (Pfizer, Groton, CT) for CP-91149, and Dr. Mar Garcia Rocha (Institute for Research in Biomedicine, Barcelona, Spain) for the antibody to GPa.

REFERENCES

1. DeFronzo RA, Ferrannini E, Hendler R, Wahren J, Felig P. Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. Proc Natl Acad Sci USA 1978;75:5173–5177
2. Bergman RN, Beir JR, Hourigan PM. Intraportal glucose infusion matched to oral glucose absorption. Lack of evidence for "gut factor" involvement in hepatic glucose storage. Diabetes 1982;31:27–35
3. Pagliassotti MJ, Cherrington AD. Regulation of net hepatic glucose uptake in vivo. Annu Rev Physiol 1992;54:847–860
4. Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 1999;48:1198–1214
5. Adkins-Marshall B, Pagliassotti MJ, Asher JR, et al. Role of hepatic nerves in response of liver to intraportal glucose delivery in dogs. Am J Physiol 1992;262:E679–E686
6. Niijima A. Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus nerve in the guinea-pig. J Physiol 1982;332:315–323
7. Shimazu T. Central nervous system regulation of liver and adipose tissue metabolism. Diabetologia 1981;20(Suppl.):343–356
8. Bollen M, Keppens S, Stalmans W. Specific features of glycogen metabolism in the liver. Biochem J 1998;336:19–31
9. Püschel GP. Control of hepatocyte metabolism by sympathetic and parasympathetic hepatic nerves. Anat Rec A Discov Mol Cell Evol Biol 2004;280:854–867
10. Jungermann K, Gardemann A, Beuers U, et al. Regulation of liver metabolism by the hepatic nerves. Adv Enzyme Regul 1987;26:63–88
11. Mondon CE, Burton SD. Factors modifying carbohydrate metabolism and effect of insulin in perfused rat liver. Am J Physiol 1971;220:724–734
12. Ottolenghi C, Cania A, Barnabei O. Effect of acetylcholine on glycogen formation and the activity of glycogen synthetase in isolated, perfused rat liver. Nature 1971;229:420–422
13. Gardemann A, Beck H, Jungermann K. Differential control of glycogenolysis and flow by arterial and portal acetylcholine in perfused rat liver. Biochem J 1990;271:599–604
14. Hampson LJ, Agius L. Acetylcholine exerts additive and permissive but not synergistic effects with insulin on glycogen synthesis in hepatocytes. FEBS Lett 2007;581:3955–3960
15. el-Salhy M, Stenling R, Grimelius L. Peptidergic innervation and endocrine cells in the human liver. Scand J Gastroenterol 1993;28:809–815
16. Lam DD, Heisler LK. Serotonin and energy balance: molecular mechanisms and implications for type 2 diabetes. Expert Rev Mol Med 2007;9:1–24

17. Moore MC, Geho WB, Lautz M, Farmer B, Neal DW, Cherrington AD. Portal serotonin infusion and glucose disposal in conscious dogs. Diabetes 2004;53: 14–20
18. Hannon J, Hoyer D. Molecular biology of 5-HT receptors. Behav Brain Res 2008;195:198–213
19. Lesurtel M, Graf R, Aleil B, et al. Platelet-derived serotonin mediates liver regeneration. Science 2006;312:104–107
20. Hampson LJ, Mackin P, Agius L. Stimulation of glycogen synthesis and inactivation of phosphorylase in hepatocytes by serotonergic mechanisms, and counter-regulation by atypical antipsychotic drugs. Diabetologia 2007; 50:1743–1751
21. Starrenburg FC, Bogers JP. How can antipsychotics cause Diabetes Mellitus? Insights based on receptor-binding profiles, humoral factors and transporter proteins. Eur Psychiatry 2009;24:164–170
22. Derijks HJ, Meyboom RH, Heerdink ER, et al. The association between antidepressant use and disturbances in glucose homeostasis: evidence from spontaneous reports. Eur J Clin Pharmacol 2008;64:531–538
23. Chan TM, Exton JH. A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal Biochem 1976;71:96–105
24. Aiston S, Hampson L, Gómez-Foix AM, Guinovart JJ, Agius L. Hepatic glycogen synthesis is highly sensitive to phosphorylase activity: evidence from metabolic control analysis. J Biol Chem 2001;276:23858–23866
25. Wilson WA, Wang Z, Roach PJ. Regulation of yeast glycogen phosphorylase by the cyclin-dependent protein kinase Pho85p. Biochem Biophys Res Commun 2005;329:161–167
26. Payne VA, Arden C, Wu C, Lange AJ, Agius L. Dual role of phosphofructokinase-2/fructose bisphosphatase-2 in regulating the compartmentation and expression of glucokinase in hepatocytes. Diabetes 2005;54:1949–1957
27. Gomis RR, Favre C, García-Rocha M, Fernández-Novell JM, Ferrer JC, Guinovart JJ. Glucose 6-phosphate produced by gluconeogenesis and by glucokinase is equally effective in activating hepatic glycogen synthase. J Biol Chem 2003;278:9740–9746
28. Hajduch E, Rencurel F, Balendran A, Batty IH, Downes CP, Hundal HS. Serotonin (5-hydroxytryptamine), a novel regulator of glucose transport in rat skeletal muscle. J Biol Chem 1999;274:13563–13568
29. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 2002;71: 533–554
30. Ismaiel AM, Titeler M, Miller KJ, Smith TS, Glennon RA. 5-HT1 and 5-HT2 binding profiles of the serotonergic agents alpha-methylserotonin and 2-methylserotonin. J Med Chem 1990;33:755–758
31. Zhang W, Bymaster FP. The in vivo effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine D1, D2, D3, 5HT2A and muscarinic receptors. Psychopharmacology (Berl) 1999;141:267–278
32. Audinot V, Newman-Tancredi A, Cussac D, Millan MJ. Inverse agonist properties of antipsychotic agents at cloned, human (h) serotonin (5-HT)(1B) and h5-HT(1D) receptors. Neuropsychopharmacology 2001;25:410–422
33. Agius L. Glucokinase and molecular aspects of liver glycogen metabolism. Biochem J 2008;414:1–18
34. Agius L. Physiological control of liver glycogen metabolism: lessons from novel glycogen phosphorylase inhibitors. Mini Rev Med Chem 2010;10: 1175–1187
35. Härndahl L, Schmoll D, Herling AW, Agius L. The role of glucose 6-phosphate in mediating the effects of glucokinase overexpression on hepatic glucose metabolism. FEBS J 2006;273:336–346
36. Bain J, Plater L, Elliott M, et al. The selectivity of protein kinase inhibitors: a further update. Biochem J 2007;408:297–315
37. Lalioti V, Pulido D, Sandoval IV. Cdk5, the multifunctional surveyor. Cell Cycle 2010;9:284–311
38. Van Schaftingen E. Involvement of phosphorylase kinase inhibition in the effect of resorcinol and proglycosyn on glycogen metabolism in the liver. Eur J Biochem 1995;234:301–307
39. Ubeda M, Kemp DM, Habener JF. Glucose-induced expression of the cyclin-dependent protein kinase 5 activator p35 involved in Alzheimer's disease regulates insulin gene transcription in pancreatic beta-cells. Endocrinology 2004;145:3023–3031
40. Li T, Chalifour LE, Paudel HK. Phosphorylation of protein phosphatase 1 by cyclin-dependent protein kinase 5 during nerve growth factor-induced PC12 cell differentiation. J Biol Chem 2007;282:6619–6628
41. Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD. Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the conscious dog. J Clin Invest 1996; 97:81–91
42. Nakamura T, Tomomura A, Kato S, Noda C, Ichihara A. Reciprocal expressions of alpha 1- and beta-adrenergic receptors, but constant expression of glucagon receptor by rat hepatocytes during development and primary culture. J Biochem 1984;96:127–136
43. Kajiyama Y, Ui M. Switching from alpha 1- to beta-subtypes in adrenergic response during primary culture of adult-rat hepatocytes as affected by the cell-to-cell interaction through plasma membranes. Biochem J 1994;303: 313–321
44. Bollen M, Peti W, Ragusa MJ, Beullens M. The extended PP1 toolkit: designed to create specificity. Trends Biochem Sci 2010;35:450–458
45. Perry JR, Frayling TM. New gene variants alter type 2 diabetes risk predominantly through reduced beta-cell function. Curr Opin Clin Nutr Metab Care 2008;11:371–377
46. Aggen JB, Nairn AC, Chamberlin R. Regulation of protein phosphatase-1. Chem Biol 2000;7:R13–R23
47. Schnack C, Hengerer B, Gillardon F. Identification of novel substrates for Cdk5 and new targets for Cdk5 inhibitors using high-density protein microarrays. Proteomics 2008;8:1980–1986
48. Liu F, Ma XH, Ule J, et al. Regulation of cyclin-dependent kinase 5 and casein kinase 1 by metabotropic glutamate receptors. Proc Natl Acad Sci USA 2001;98:11062–11068
49. Benavides DR, Bibb JA. Role of Cdk5 in drug abuse and plasticity. Ann N Y Acad Sci 2004;1025:335–344
50. Dicostanzo CA, Dardevet DP, Neal DW, et al. Role of the hepatic sympathetic nerves in the regulation of net hepatic glucose uptake and the mediation of the portal glucose signal. Am J Physiol Endocrinol Metab 2006; 290:E9–E16