Nanomolar Melatonin Influences Insulin Synthesis and Secretion in Rat Insulinoma INS-1E Cells

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Research

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Abstract

Background: Pancreatic beta-cell dysfunction results in reductions of insulin synthesis/secretion, cell survival, and insulin sensitivity thereby inducing diabetes mellitus. In this study, how nanomolar melatonin regulates insulin synthesis and secretion in rat insulinoma INS-1E cells was investigated.

Methods: Western Blot and ELISA were performed to investigate the insulin synthesis and secretion at melatonin concentrations of 10-100 nM for 48 h and 10-500 nM for 72 h in rat insulinoma INS-1E cells.

Results: Melatonin significantly increased the insulin protein level in INS-1E cells above the level in control cells without melatonin or glucose treatments and decreased the insulin protein level in media with glucose. At melatonin concentrations of 10-100 nM for 48 h and 10-500 nM for 72 h, increases and decreases occurred in dose-dependent manners. Luzindole or 4-phenyl-2-propionamidotetralin (4P-PDOT), melatonin receptor (MR) antagonists, inhibited the melatonin-induced insulin protein level in cells and media. Levels of membrane vesicle trafficking-related proteins including Rab5, GOPC, and phospho-caveolin-1 proteins significantly increased with melatonin treatment above that in control cells without melatonin or glucose treatments, whereas expressions of EEA1, clathrin, APPL1, and syntaxin-6 proteins significantly decreased with melatonin treatment. The increases in the phosphorylation of the mammalian target of rapamycin (p-mTOR) and Raptor protein levels (mTOR Complex 1 (mTORC1)) were consistent with the increments in the expressions of p-Akt (Ser473, Thr308). Also, expression levels of Bcl-2 and Bcl-xL proteins were significantly increased compared to those in control cells without melatonin or glucose treatments, whereas the Bax protein level decreased. Luzindole or 4P-PDOT decreased the melatonin-induced levels of p-mTOR (Ser2448), Raptor, p-Akt (Ser473), and Bcl-2 proteins, whereas MR antagonists increased the melatonin-suppressed level of Bax proteins.

Conclusion: These results indicate that nanomolar melatonin regulates insulin synthesis and secretion associated with membrane vesicle trafficking-related proteins, including Rab5, GOPC, p-Caveolin-1, EEA1, and clathrin through the Akt/mTORC1 pathway.

Background

Hyperglycemia, dyslipidemia, inflammation, autoimmunity, islet amyloid, and insulin resistance influence the functioning of pancreatic beta cells. Pancreatic beta-cell dysfunction results in reductions of insulin synthesis/secretion, cell survival, and insulin sensitivity, thereby inducing diabetes mellitus [1, 2]. Decreases in insulin production/secretion related to diabetes and obesity have been associated with a reduction of β cell mass from apoptosis in animal models and human patients [3, 4].

Vesicle trafficking includes the intra- and extracellular transport processes of exocytosis and endocytosis performed by membrane vesicles and involved with the membrane-associated proteins clathrin, caveolin, Rab proteins, soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNAREs), synaptotagmin, synaptophysin, and others. Caveolins are involved in cholesterol and vesicular trafficking, endothelial cell function, and human diseases including diabetes, atherosclerosis, cardiac hypertrophy,

pulmonary hypertension, cardiomyopathy, and muscular dystrophy [5–7]. Clathrin has a significant role in coated vesicles for intracellular membrane trafficking. Clathrin-coated vesicles selectively sort cargo at the plasma membrane of the cell, the trans-Golgi membrane, and at endosomal compartments for multiple membrane trafficking [8]. Clathrin is not implicated in proinsulin sorting within the trans-Golgi network, the formation of β-cell secretory granules, proinsulin conversion, or the regulation of proinsulin or insulin exocytosis [9]. Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) is involved in endosomal vesicle trafficking and the GPCR/Akt signaling pathway [10–13]. In pancreatic β cells, APPL1 induces a reduction in expression of the exocytotic machinery-related SNARE proteins and it reduces insulin secretion through Akt activation [10]. Also, APPL1 regulates beta-cell mitochondrial structure and function [14]. INS-1 β-cells induce the expression of membrane-anchorless, cytosolic syntaxin-6 that is involved in endosomal functioning [15]. Early endosomal antigen 1 (EEA1) requires angiotensin II-induced activation/phosphorylation of Akt in the early endosomes of vascular smooth muscle cells [16]. Insulin activates Rab5, which leads to phosphatidylinositol 3-phosphate formation at the plasma membrane [17].

A physiological or nanomolar concentration of melatonin induces mitochondrial bioenergetic effects within metabolic pathways [18]; moreover, such concentrations promote the improvement of microgravity-related bone loss or osteoporosis [19]. However, Sáenz et al. [20] reported that low concentrations (1 pM-10 nM) of melatonin significantly decrease the retinal nitrergic pathway in the Syrian hamster retina. A previous study demonstrated that a pharmacological concentration (10, 50 μM) of melatonin mediates insulin synthesis via RNA-binding protein human antigen D (HuD) protein expression in rat INS-1E cells [21]. Several other researchers have reported that various concentrations of melatonin can regulate insulin secretion [22–25]. Especially, Peschke´s group show that 100 nM melatonin influence insulin secretion in media of INS-1 cells [22, 23]. However, it is required how a physiological melatonin concentration can directly influence intracellular biosynthesis and extracellular secretion of insulin. Therefore, the present study investigated the nanomolar melatonin regulation of insulin synthesis and secretion involving with vesicle trafficking-related proteins, ER stress, Bcl-2/Bax, and Akt/mTORC1 in rat insulinoma INS-1E cells.

Materials And Methods

Cell culture

The INS-1E cells, a clonal pancreatic β-cell line received from Prof. Claes B. Wollheim (Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland), were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) containing 11 mM glucose supplemented with 10 mM HEPES (pH 7.3), 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), 50 μM β-mercaptoethanol, 1 mM sodium pyruvate, 50 μg/mL penicillin, and 100 μg/mL streptomycin at 37°C with 5% CO_2 in a humidified incubator. The INS-1E cells on 100 mm culture plate (Corning, Corning, NY, USA) at a density of 8×10^6 were cultured in RPMI 1640 medium plus 2% heat-inactivated FBS with/without melatonin (10, 50, 100

nM) (Calbiochem, San Diego, MO, USA) in the presence/absence of 10 μM luzindole (Calbiochem) or 10 μM 4-phenyl-2- propionamidotetralin (4P-PDOT) (Sigma-Aldrich, St. Louis, MO, USA) at 37°C with 5% CO_2 for 48 or 72 h.

Western blot analysis

Cells were harvested and resuspended in 20 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, 5 μg/mL pepstatin A, 1 μg/mL chymostatin, 5 mM Na_3VO_4 and 5 mM NaF. The whole cell lysate was followed by centrifugation at 13,000 × *g* for 10 min at 4°C. Protein concentrations were determined by using the BCA assay (Sigma, St Louis, MO, USA). Proteins (40 μg) or media (20 μL) were separated via 10% Gradi-Gel II gradient PAGE (ELPIS-Biotech, Taejeon, Republic of Korea) and then transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated with antibodies: insulin (1:500; sc-9168; Santa Cruz Biotechnology, Santa Cruz, CA, USA); Rab5 (1:1000; #2143; Cell Signaling Technology, Beverly, MA, USA); GOPC (1:1000 dilution; #4646; Cell Signaling Technology); p-caveolin-1 (1:1000; #3251; Cell Signaling Technology); caveolin-1 (1:1000; #3267; Cell Signaling Technology); EEA1 (1:1000; #3288; Cell Signaling Technology); clathrin (1:1000; #4796; Cell Signaling Technology); APPL1 (1:1000; #3858; Cell Signaling Technology); syntaxin-6 (1:1000; #2869; Cell Signaling Technology); p-mTOR (Ser2448; 1:1000; #2971; Cell Signaling Technology); p-mTOR (Ser 2481; 1:1000; #2974; Cell Signaling Technology); mTOR (1:1000; #2983; Cell Signaling Technology); Raptor (1:1000; #2280; Cell Signaling Technology); Rictor (1:1000; #2114; Cell Signaling Technology); p-Akt (Thr 308; 1:1000; #9275; Cell Signaling Technology); p-Akt (Ser 473; 1:1000; #9271; Cell Signaling Technology); Akt (1:500; sc-8321; Santa Cruz Biotechnology); p-eIF2α (1:1000; #3597; Cell Signaling Technology); eIF2α (1:1000; #5324; Cell Signaling Technology); IRE1α (1:1000; #3294; Cell Signaling Technology); GRP78/BiP (1:1000; #3177; Cell Signaling Technology); Bcl-2 (1:500; sc-7382; Santa Cruz Biotechnology); Bcl-xL (1:1000; #2762; Cell Signaling Technology); Bax (1:500; sc-7380; Santa Cruz Biotechnology); and β-actin (1:500; sc-81178; Santa Cruz Biotechnology). The membrane was incubated with Anti-rabbit and anti-mouse IgG conjugated horseradish peroxidase secondary antibodies (Santa Cruz Biotechnology), and then with ECL Western-blotting reagents (Pierce Biotechnology, Rockford, IL, USA). Immunoreactive proteins were visualized by exposure to X-ray film. Protein bands were visualized by image-scanning, and optical density was measured by using ImageJ analysis software (version 1.37; Wayne Rasband, NIH, Bethesda, MD, USA) after the data were corrected by background subtraction and normalized by including β-actin as an internal control.

Measurement of insulin protein by ELISA

The insulin levels were determined by using the insulin ELISA kit (ALPCO Diagnostics, Salem, NH, USA) according to the manufacturer's instructions. Serially diluted standards, samples, and the conjugate of horseradish peroxidase-labeled insulin monoclonal antibody were added to the 96-well microplate coated with insulin antibody and incubated at 700 × *g* on a horizontal shaker for 2 h at room temperature (RT).

Any residual conjugate was removed by washing 6 times with Working Strength Wash buffer and the wells were incubated with TMB substrate at RT for 15 min. The reaction was stopped by adding a stop solution. Absorbance was measured at 450 nm. This assay was calibrated against standards.

Statistical analysis

Significant differences were detected by using ANOVA followed by Tukey's test for multiple comparisons. The analysis was performed using the Prism Graph Pad v4.0 (Graph Pad Software, San Diego, CA, USA). Values are expressed as means ± SEM of at least three separate experiments, in which case a representative result is depicted in the figures. *P* values < 0.05 were considered statistically significant.

Results

Initially, we investigated nanomolar melatonin concentration-related regulation of insulin synthesis in cells and insulin secretion in media over time. Melatonin significantly increased the insulin protein level in cells compared to that in control cells without melatonin or with glucose treatment and decreased the insulin level in media compared to that in media with glucose treatment; both results occurred in a dose-dependent manner at melatonin concentrations from 10 nM to 100 nM when treated for 48 h (Fig. 1a, b). This increase in insulin concentration was demonstrated with ELISA (Fig. 1c). Besides, 10–500 nM melatonin treatments for 72 h significantly decreased the insulin protein level in cells in a dose-dependent manner and decreased the insulin level in media from that with glucose treatment (Fig. 1a, b). This means that the results of 72 h incubation are opposite to that of 48hr regarding the dose-dependent effect. Both of 10 μM Luzindole, melatonin receptor 1, 2 antagonists, and 10 μM 4P-PDOT, melatonin receptor 2 antagonist, inhibited the melatonin-induced increase in insulin levels in cells and media (Fig. 2).

Next, we demonstrated the effect of nanomolar melatonin on insulin synthesis and secretion involving vesicle trafficking-related proteins, Bcl-2/Bax, and Akt/mTOR in INS-1E cells. Levels of membrane vesicle trafficking-related proteins including Rab5, GOPC, and phospho-caveolin-1 significantly increased with nanomolar melatonin treatment when compared to the levels in the control cells without melatonin or glucose treatments (Fig. 3a, b), whereas expressions of EEA1, clathrin, APPL1, and syntaxin-6 proteins significantly decreased with nanomolar melatonin treatment (Fig. 3a, c, d). mTOR Complex 1 (mTORC1) consists of two proteins, phosphorylation of mammalian target of rapamycin (p-mTOR, S2448) and rapamycin-sensitive adaptor protein of mTOR (Raptor). p-mTOR (S2448) and raptor protein, significantly increased with 10 nM to 100 nM melatonin treatments compared to those with glucose treatment (Fig. 4a, b); in contrast, the Rictor protein level decreased with 10 nM to 100 nM melatonin treatment (Fig. 4a, c). The observed increments in p-Akt levels (Ser473, Thr308) were consistent with the mTORC1 result (Fig. 5). This result indicated that nanomolar melatonin influences insulin synthesis and secretion under the Akt/ mTORC1 pathway.

The expression levels of Bcl-2 and Bcl-xL proteins significantly increased compared to those in control cells without melatonin or glucose treatments, whereas the level of Bax protein decreased (Fig. 6).

We identified the effect of MR antagonists on the Akt/mTORC1 pathway, Bcl-2, and Bax. Luzindole or 4P-PDOT significantly decreased the melatonin-induced levels of p-mTOR (Ser2448), Raptor, p-Akt (Ser473), and Bcl-2 proteins (Fig. 7). These results indicate that nanomolar melatonin concentrations can increase the insulin protein level in cells and decrease that level in media through regulation of vesicle trafficking-related proteins, Bcl-2/Bax, and Akt/mTORC1 pathway.

Discussion

The daily insulin secretion pattern correlates with the circadian rhythm including melatonin in pancreatic islets and beta cells [26, 27]. Melatonin suppresses insulin secretion in both rats and mice [28–31]. Moreover, melatonin can have direct or indirect effects under physiological conditions. Such differences could be attributed to the different time courses for melatonin administration and the requirements for daily rhythmicity. In this condition, melatonin has direct effects on insulin secretion in pancreatic islets and beta cells, effects that can be dependent on melatonin receptors. The non-hydrolyzable GTP analog guanosine 5'-O-(3-thiotriphosphate) and the melatonin antagonist luzindole block the effects of melatonin on insulin secretion in neonatal rat islets [32]. INS-1 cells [33, 34], rat islets [35, 36], and human islets [36] express *MTNR1A* mRNA. Also, *MTNR1B* mRNA has been detected in rat [35, 36] and human [36] islets. MIN-6 cells express both forms of the melatonin receptor [35]. The present study showed that 10–100 nM melatonin concentrations can increase the insulin level in cells and decrease the insulin level in media in a melatonin dose-dependent manner and with dependence on melatonin receptors.

Physiological melatonin concentrations lower than nanomolar concentrations are involved with the regulation of many biological and physiological processes in the human body and are dependent on melatonin receptor functions within the gastrointestinal system, heart, brain, and immune system [37, 38]. In vitro, nanomolar melatonin can directly suppress human breast cancer cell survival through the estrogen-response pathway, as well as a decrease in the activity and expression of aromatase, sulfatase, and 17 beta-hydroxysteroid dehydrogenase, and an increase in the activity and expression of estrogen sulfotransferase. These observations support the suggestion that melatonin is mainly associated with antiestrogenic actions and interactions in tumor cells' estrogen-signaling pathway [39]. Arese et al. [18] demonstrated that a 1 nM concentration of melatonin in HaCaT cells incubated for 3–6 h induces a 4-fold increase in neuronal NOS mRNA expression above the basal level. HaCaT cells exposed to melatonin concentrations in the 1 nM range for 5 h showed the entry of melatonin into cells and heterogeneous distribution of melatonin levels among organs and tissues, resulting in a cell melatonin concentration of $52.86 \times 10^{-3} \pm 0.0063$ pM, which is similar to the melatonin concentration flowing in the human blood at night (approximately 100–200 pg/mL), the peak melatonin concentration-time [40, 41]. Melatonin treatment at 1 pM to 1 nM showed a nitrergic inhibition in hamster retina [20], suggesting that melatonin

probably modulates the nitrergic activity through a receptor-mediated mechanism in the retina. This effect of melatonin on the nitrergic activity was shown to be dependent on the physiological concentration in rat hypothalamus and cerebellum [42, 43]. Melatonin, a highly lipophilic molecule, can enter cells and cross all physiological barriers. However, this effect of melatonin can be restricted to cell types that express both melatonin receptors and nitrergic activity [44]. The present study demonstrated that nanomolar melatonin concentrations can influence insulin synthesis in rat insulinoma INS-1E cells and insulin secretion in media, which actions are dependent on melatonin receptors.

This study demonstrated the insulin secretion and vesicle trafficking-related proteins include Rab5, GOPC, caveolin-1, EEA1, clathrin, APPL1, and syntaxin-6. In an early endosome fusion, GOPC interacts with syntaxin- 6 [45], and syntaxin-6 is a member of the syntaxin family of SNAREs that are Rab5 effectors. This family comprises at least 17 members, all of which localize to membrane compartments along the endocytic and exocytic pathways [46, 47]. Syntaxin-6 can interact with the EEA1 protein, a Rab5 effector [48], suggesting that syntaxin-6 may promote the tethering of post-Golgi vesicles to early endosomes. Cytosolic syntaxin-6 has predominantly negative effects on the endosomal system of pancreatic β-cells and can provide indirect interference with insulin secretory pathways [15]. The APPL1 protein leads to the phosphatidylinositol 3-kinase (PI3K)-dependent formation of EEA1-positive early endosomes [49] which activate the Rab5 effector [50, 51]. APPL1 protein regulates insulin secretion in pancreatic β cells by regulating the expression of SNARE proteins through the PI3K/Akt-dependent pathway, suggesting that APPL1 protein is a master coordinator and controller of insulin secretion [10]. Clathrin, a cargo protein of transport vesicles, is not required in the formation of β-cell secretory granules or to process or regulate exocytosis of proinsulin/insulin [9].

The key target of Akt/PKB and insulin in a multitude of cellular events including glucose uptake, glycogen synthesis, gluconeogenesis, and lipid storage is phosphoinositide 3-kinase (PI3K) to specific sites on IRS1/IRS2 that are tyrosine-phosphorylated by the insulin receptor [21, 52–55]. A major down-regulator of insulin action is mTOR, which belongs to the PI3K-related kinase protein family. mTOR functions in a mitogenic pathway downstream of PI3K and is activated by insulin and growth factors in the presence of sufficient nutrients including amino acids and glucose [56, 57]. mTOR Complex 1 (mTORC1) is composed of rapamycin-sensitive adaptor protein of mTOR (Raptor), G-protein β-subunit-like protein (GβL), and the Akt/ PKB substrate 40 kDa (PRAS40) [58, 59]. The mTORC1 is activated by insulin, growth factors, serum, phosphatidic acid, amino acids (particularly leucine), and oxidative stress [58, 60]. mTOR Complex 2 (mTORC2) is composed of mTOR, rapamycin-insensitive companion of mTOR (Rictor), GβL, and mammalian stress-activated protein kinase interacting protein 1 (mSIN1) [61, 62]. mTORC2 phosphorylates the Akt/PKB at serine 473 residue. Phosphorylation of serine stimulates Akt phosphorylation at threonine 308 residue by PDK1 and leads to full Akt activation [63, 64]. It has been reported that mTORC2 is regulated by insulin, growth factors, serum, and nutrient levels [61]. The present study shows that insulin biosynthesis and secretion through the Akt/mTORC1 pathway.

The Bcl-2 family proteins including Bcl-2, Bcl-xL, and Bax have been studied as a pro- and antioxidant [62–64]. The localization of Bcl-2 to the mitochondria indicates it has a role in generating a slight pro-

and antioxidant state via mitochondrial machinery [65, 66]. Chen and Pervaiz [67] proposed that mitochondrial respiration, as a novel mechanism, can control survival even in the presence of ROS, indicating that this protective mechanism, including antioxidant defense systems such as SOD, can prevent ROS production. Mn-SOD, localized to the mitochondrial matrix, predominantly quenches mitochondrial O^{2-} and increases activity through Bcl-2 overexpression. In this study, the expression levels of Bcl-2, Bcl-xL, and Bax proteins involved in the regulation of insulin synthesis or its secretion from β-cells.

Conclusion

This study shows that intracellular production and extracellular secretion of insulin is increased after 48 h of incubation with 10–100 nM concentrations of melatonin. The increases are associated with membrane vesicle trafficking-related proteins, including Rab5, GOPC, p-Caveolin-1, EEA1, and clathrin, involved in the Akt/mTORC1 pathway.

Abbreviations

4P-PDOT: Luzindole or 4-phenyl-2-propionamidotetralin; p-mTOR: phosphorylation of mammalian target of rapamycin; mTORC1: mTOR complex 1; ER: endoplasmic reticulum; SNAREs: soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins; APPL1: adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; EEA1: early endosomal antigen 1; NOS: nitric oxide synthase; PI3K: phosphatidylinositol 3-kinase; Raptor: rapamycin-sensitive adaptor protein of mTOR; Rictor: rapamycin-insensitive companion of mTOR.

Declarations

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

All relevant data are available in this published paper.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author’s Contributions

EMJ and YMY wrote the paper. EMJ, SSJ and YMY designed the experiments and performed and analyzed the data. SSJ oversaw the project. All authors read and approved the final manuscript.

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Figures

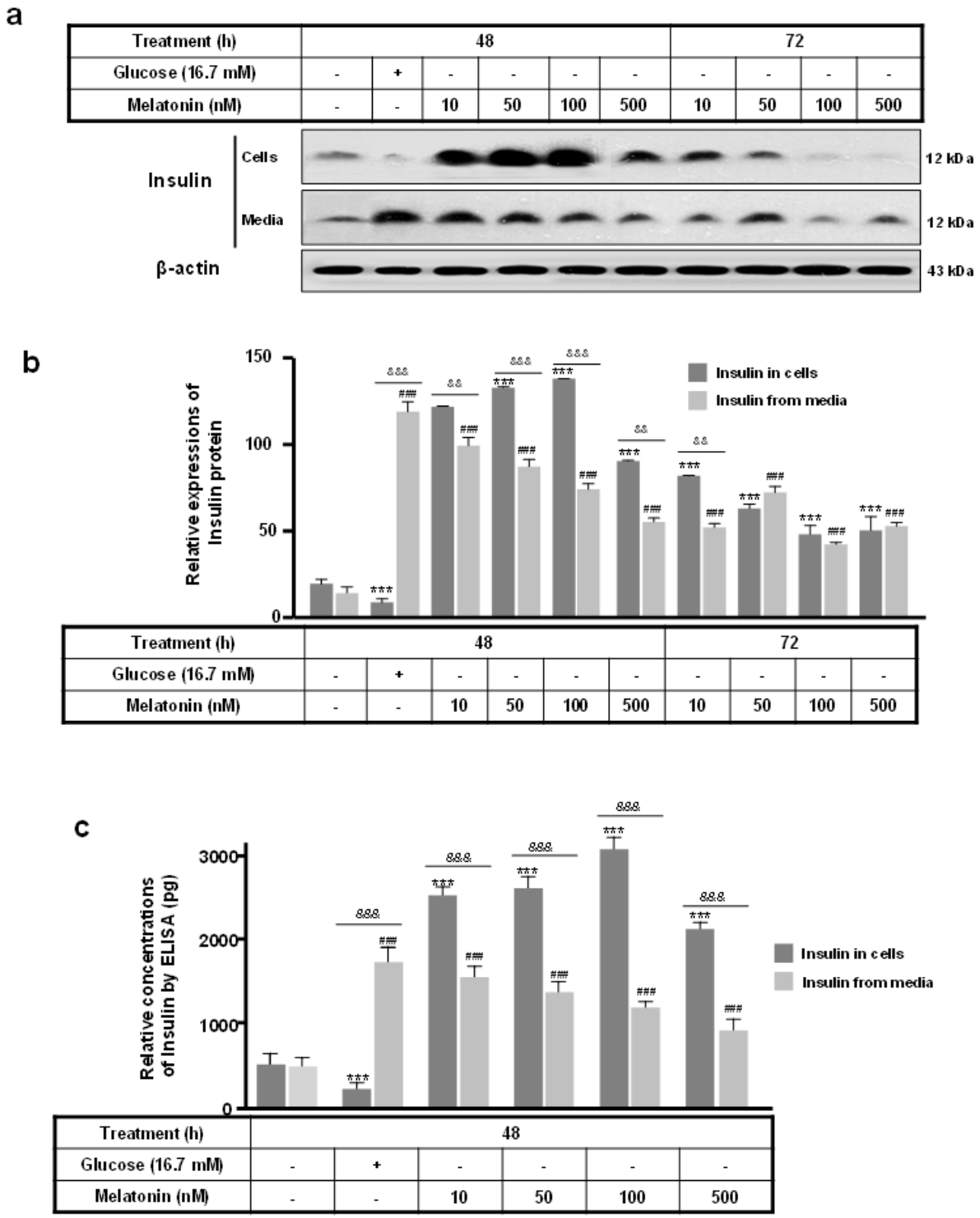

Figure 1

The expressions of insulin protein in cells and media under melatonin treatment in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% with melatonin for 48 and 72 h at 37°C with 5% CO2. Insulin proteins were then analyzed by Western blot (a). The relative amounts of insulin protein (b) were quantified as described in Materials and methods. Insulin concentration was measured by ELISA (c). Data represent the mean ± SEM of three experiments. ***p <

0.001 vs. 2% FBS; ###p < 0.001 vs. 2% FBS; &&p < 0.01, &&&p < 0.001, insulin in cells vs. insulin in media; %%%p < 0.001 vs. Glucose. Scale bar, 100 μm.

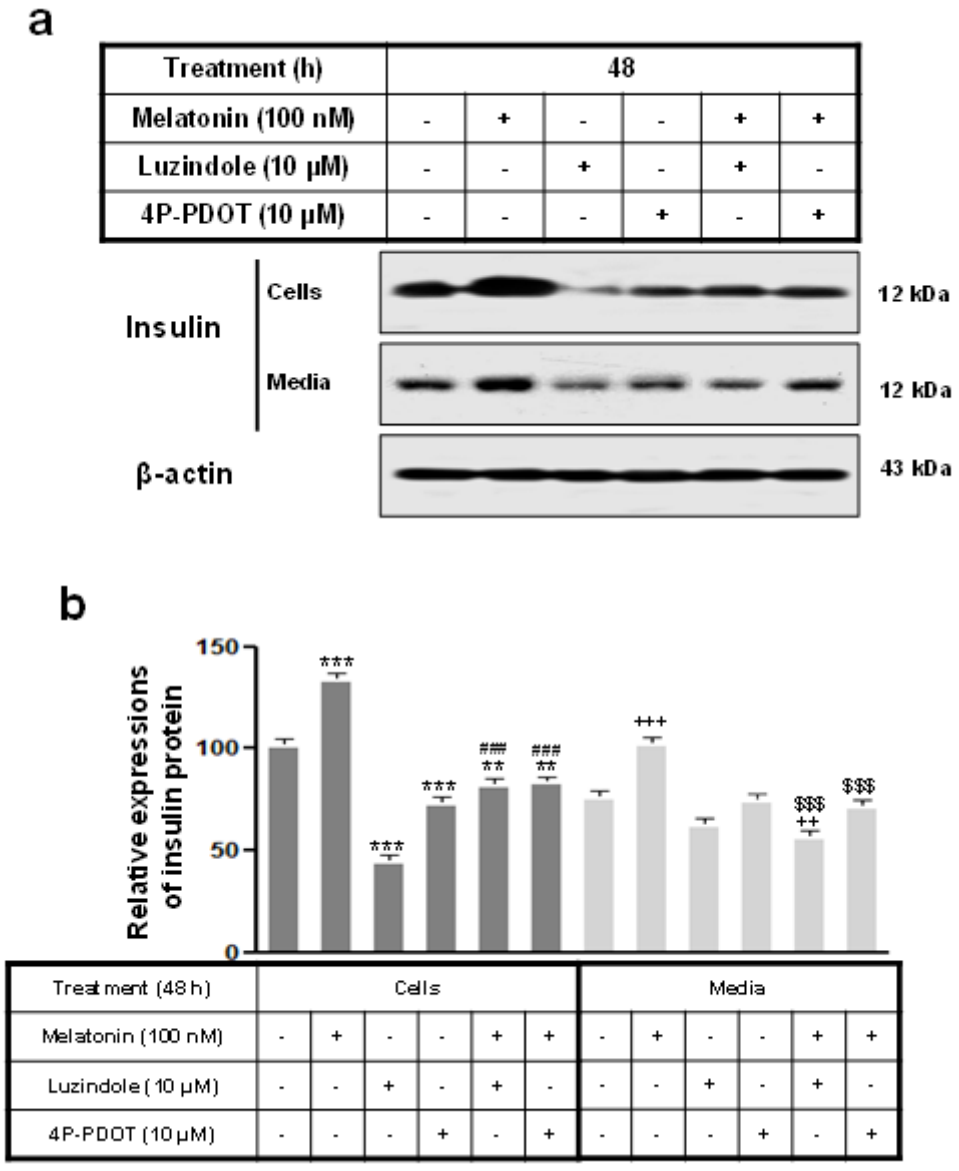

Figure 2

The expressions of insulin protein in cells and media under melatonin and/or luzindole/4P-PDOT treatment in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% FBS under melatonin (100 nM) and/or luzindole (10 μM) or 4P-PDOT (10 μM) treatments for 48 h at 37°C with 5% CO2. Insulin proteins were then analyzed by Western blot (a). The relative amounts of insulin protein (b) were quantified as described in Materials and methods. Data represent the mean ± SEM of three experiments. **p < 0.01, ***p < 0.001 vs. 2% FBS; ###p < 0.001 vs. melatonin; ++p < 0.01, +++p < 0.001 vs. 2% FBS in media; $$$p < 0.001 vs. melatonin in media.

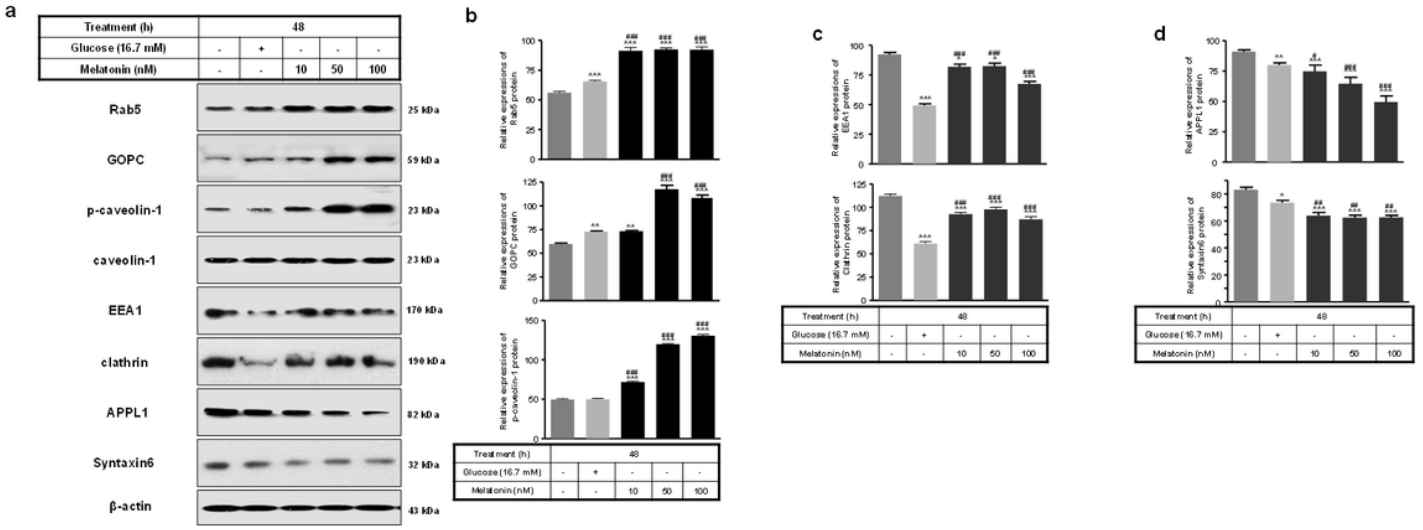

Figure 3

Levels of membrane vesicle trafficking-related proteins including Rab5, GOPC, p-caveolin-1, EEA1, clathrin, APPL1, and Syntaxin6 in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% with melatonin for 48 h at 37°C with 5% CO2. The expressions of membrane vesicle trafficking-related proteins were then analyzed by Western blot (a). The relative amounts of Rab5 (b), GOPC (b), p-caveolin-1 (b), EEA1 (c), clathrin (c), APPL1 (d), and Syntaxin6 (d) were quantified as described in Materials and methods. Data represent the mean ± SEM of three experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs. 2% FBS; #p < 0.05, ##p < 0.01, ###p < 0.001, glucose vs. melatonin.

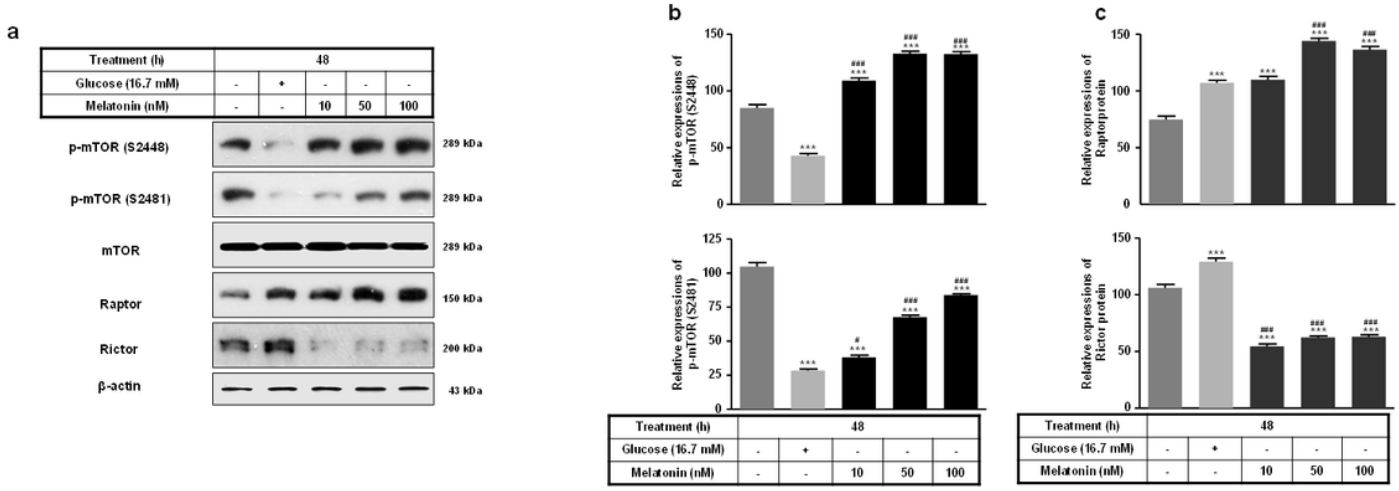

Figure 4

The expressions of p-mTOR (Ser2448, Ser2481), Raptor, and Rictor proteins under melatonin treatment in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% FBS with melatonin for 48 h at 37°C with 5% CO2. p-mTOR (Ser2448, Ser2481), Raptor, and Rictor

proteins were then analyzed by Western blot (a). The relative amounts of p-mTOR (Ser2448, Ser2481) (b) Raptor, and Rictor (c) proteins were quantified as described in Materials and methods. Data represent the mean ± SEM of three experiments. ***p < 0.001 vs. 2% FBS; #p < 0.05, ###p < 0.001, glucose vs. melatonin.

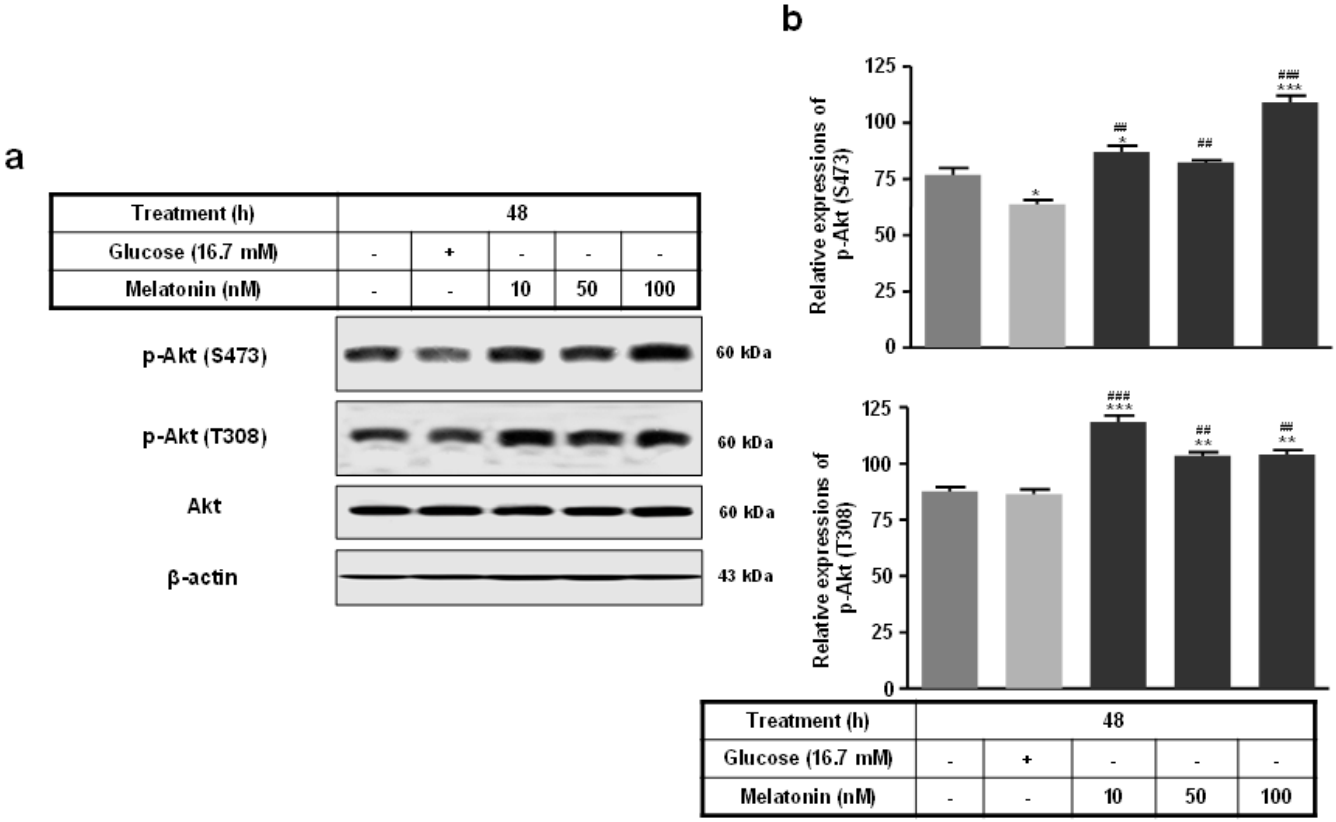

Figure 5

Phosphorylations of Akt (Ser473) and Akt (Thr308) proteins under melatonin treatment in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% FBS with melatonin for 48 h at 37°C with 5% CO2. p-Akt expression was then analyzed by Western blot (a). The relative amounts of p-Akt (Ser473) and p-Akt (Thr308) (b) were quantified as described in Materials and methods. Data represent the mean ± SEM of three experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs. 2% FBS; ##p < 0.01, ###p < 0.001, glucose vs. melatonin.

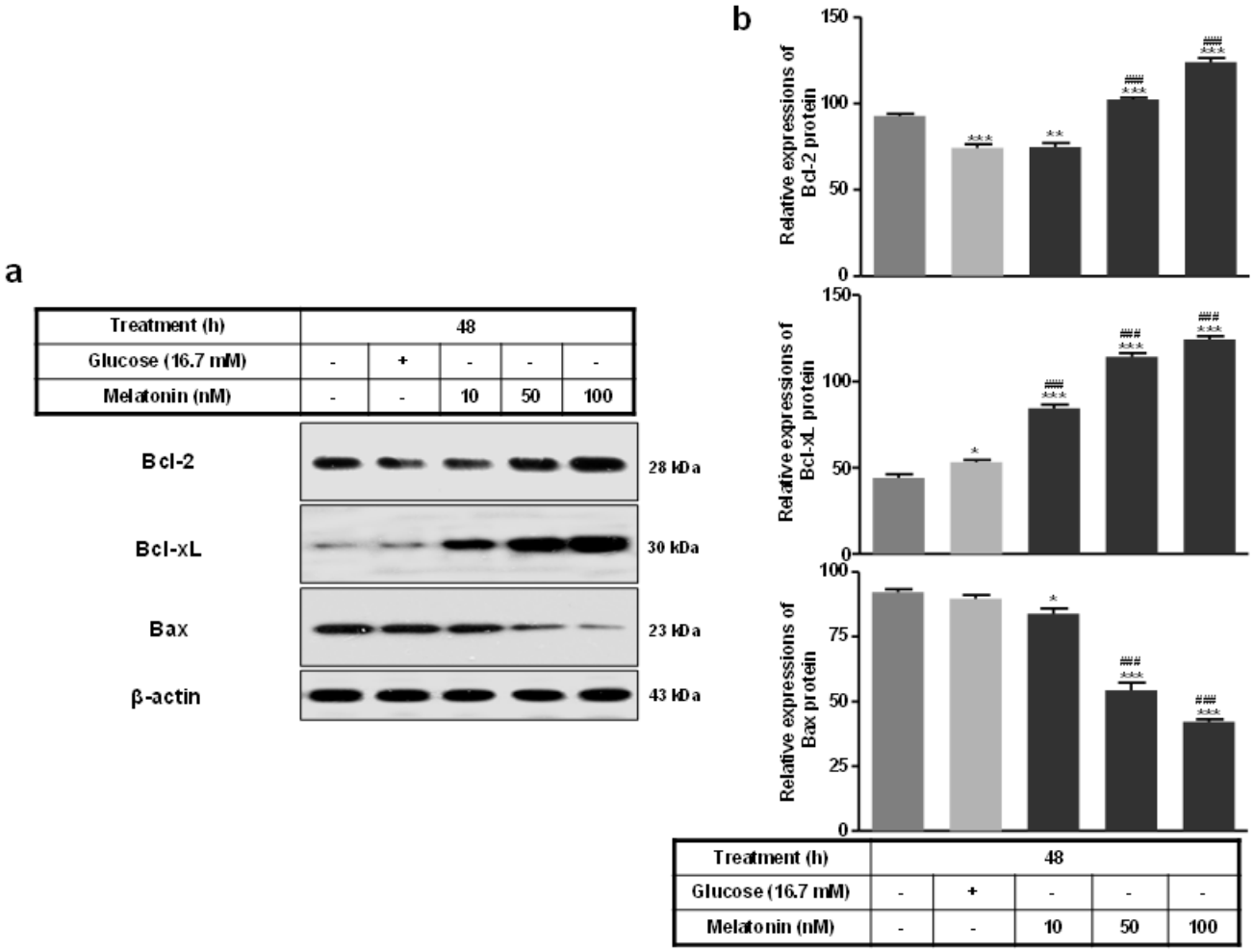

Figure 6

The expressions of Bcl-2, Bcl-xL, and Bax proteins under melatonin in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% FBS with melatonin for 48 h at 37°C with 5% CO2. Expressions of Bcl-2, Bcl-xL, and Bax proteins were then analyzed by Western blot (a). The relative amounts of BCl-2, Bcl-xL, and Bax (b) were quantified as described in Materials and methods. Data represent the mean ± SEM of three experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs. 2% FBS; ###p < 0.001, glucose vs. melatonin.

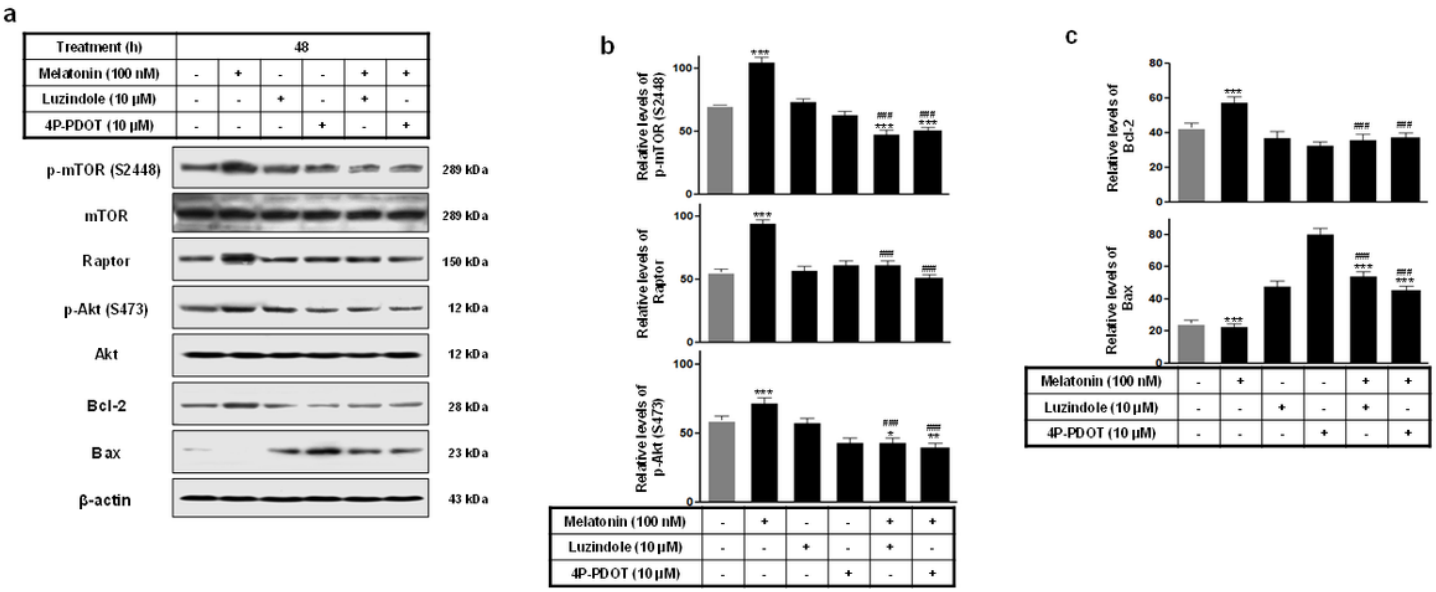

Figure 7

The expressions of p-mTOR (Ser2448), Raptor, p-Akt (Ser473), IRE1α, GRP78/BiP, Bcl-2, and Bax proteins under melatonin and/or luzindole/4-phenyl-2- propionamidotetralin treatment in rat insulinoma INS-1E cells. INS-1E cells were incubated in RPMI 1640 medium supplemented with 2% FBS under melatonin (100 nM) and/or luzindole (10 μM) or 4P-PDOT (10 μM) treatments for 48 hr at 37°C with 5% CO2. Proteins were then analyzed by Western blot (a). The relative amounts of proteins were quantified as described in Materials and methods as follows: p-mTOR (Ser2448), Raptor, p-Akt (Ser473) (b), Bcl-2 and Bax (c). Data represent the mean ± SEM of three experiments. **p < 0.01, ***p < 0.001 vs. 2% FBS; ###p < 0.001 vs. melatonin; ++p < 0.01, +++p < 0.001 vs. 2% FBS in media; $$$p < 0.001 vs. melatonin in media.