The role of circadian rhythm in the regulation of cellular protein profiles in the brain

Abstract

Background/aim: Circadian rhythm plays a significant role in the regulation of almost all kinds of physiological processes. Besides this it may also have a direct or indirect effect on the neurodegenerative processes, including Alzheimer's disease, Parkinson's disease, and ischemic stroke. Therefore, the identification of circadian rhythm related proteins is crucial to be able to understand the molecular mechanism of the circadian rhythm and to define new therapeutic target for the treatment of degenerative disorders.

Materials and methods: To identify the light and dark regulated proteins, 8-12 weeks, male Balb/C mice were used at two different time points (Morning (Zeitgeber time-0 (ZT0)) and midnight (ZT18)) under physiological conditions. Therefore, brain tissues were analyzed via liquid chromatography tandem-mass spectrometry.

Results: A totally of 1621 different proteins were identified between ZT0 and ZT18 mice. Among these proteins, 23 proteins were differentially expressed (p<0.05 and fold change 1.4) in ZT18 mice, 11 upregulated (AKAP10, ALDOC, BLK, NCALD, NFL, PDE10A, PICAL, PSMB6, RL10, SH3L3, and SYNJ1), and 12 downregulated (AT2A2, AT2B1, CPNE5, KAP3, MAON, NPM, PI51C, PPR1B, SAM50, TOM70, TY3H, and VAPA) as compared with ZT0 mice.

Conclusion: Taken together, here we identified circadian rhythm related proteins, and our further analyzed revealed that these proteins play significant roles in molecular function, membrane trafficking, biogenesis, cellular process, metabolic process, and neurodegenerative disorders such as Parkinson’s disease.

Key words: Circadian rhythm, lc-ms/ms, PDE10A, proteomics, zeitgeber time
1. Introduction

Circadian rhythm is not only regulating our sleep-wake cycle also controls almost all kinds of physiological conditions in our bodies such as body temperature, hormone secretion, immune regulation, and heart rate [1, 2]. Substantial evidence indicates that almost every mammalian cell has a circadian clock mechanism. Although there are a large number of cellular clocks in the human body, the principal circadian pacemaker in mammals is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [3, 4]. Many essential neuronal pathways, including retino-hypothalamic tract transmit light information from the eyes to the SCN to synchronize endogenous clockwork to the external environment.

At the molecular level, circadian locomotor output cycles protein kaput (CLOCK) and its heterodimer partner brain and muscle ARNT-like 1 (BMAL1) protein are the core clock proteins which drive daytime expression of period (PER) and cryptochrome proteins (CRY). In a negative transcriptional feedback loop, core clock proteins BMAL1/CLOCK regulates PER and CRY protein expression, which allows the circadian cycle to start again. Also, Bmal1 and CLOCK heterodimer complex regulate the nuclear receptor family of intracellular transcription factors REV-ERBs and RORs proteins [5].

The circadian rhythm has an essential role in the regulation of physiological activities as well as in the pathophysiological processes. In a recent study, it was demonstrated that most of the ischemic stroke cases occurs in the morning, and they suggested that the occurrence of stroke showed a circadian distribution [6]. In a previous study, we have demonstrated that time-of-day dependent protein expression plays crucial roles in the
severity of brain injury after ischemic stroke in mice [7]. In the same study, we also showed that midnight ischemia-reperfusion injury in mice resulted in significantly improved neurological behavior, neuronal survival, and reduced disseminated neuronal injury, brain edema and infarct volume compared with ischemia induced at morning time [7]. Moreover, most prevalent neurodegenerative diseases including Alzheimer’s disease, Parkinson’s disease and Huntington disease, may cause abnormalities in the circadian rhythm [8].

Due to its important role in almost all physiological conditions as well as in pathophysiological processes, circadian rhythm needs to be studied in a more detail. In this context, here we investigated the time-dependent protein expression from mice striatum via liquid chromatography tandem-mass spectrometry (LC-MS/MS) which offers new perspectives in studying biological processes under physiological conditions, which also reveal us new therapeutic targets for the development of neurodegenerative disorders.

2. Materials and Methods

2.1. Ethics Statement for animal experiments

This study has been conducted in accordance with the ethical standards according to the Declaration of Helsinki and according to national and international guidelines and has been approved by the Ethics Committee of Istanbul Medipol University (Reference number: 22/01/2020-09). All animals were maintained under a constant 12-h light/dark cycle (lights on at 06:00 daily).
2.2. **Experimental groups**

Experiments were performed using 8-12 weeks Balb/c male mice. All animals were maintained under a constant 12:12-h light-darkness regimen with ad libitum access to food and water. A total 14 animals were divided into two main groups depend on their Zeitgeber time (ZT: ZT0 is lights on, and ZT12 is lights off); ZT0 and ZT18. To evaluate time dependent protein expression at morning (n=7 mice) and midnight (n=7 mice) animals were sacrificed. In this set, protein profiling was performed using liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis.

2.3. **Sample preparation for liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis**

Animals were sacrificed at specified times (zeitgeber times (ZT) 0 or 18). Therefore, the brains were immediately removed, frozen on dry ice and stored -80°C. The brain tissues were taken from striatum and were homogenized in 50 mM ammonium bicarbonate and lysed by heating at 95 °C in protein extraction reagent kit (UPX Universal; Expedon). Samples were incubated an hour at 4 °C. After incubation step, samples were centrifuged at 14,000 G for 10 min. Then supernatants were transferred into a microcentrifuge tube. Protein concentration of samples were determined via Qubit 3.0 Fluorometer (Q33216, Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer’s protocol. As previously reported, FASP (Filter Aided Sample Preparation) Protein Digestion Kit (ab270519, Abcam) was used for generating tryptic peptides according to the manufacturer's protocol [9]. Briefly, tryptic peptides were generated according to the Filter Aided Sample Preparation Protocol (FASP). A total of 50 µg protein samples were filtered using 6M urea in a 30 kDa cut-off spin column. After
this step, samples were alkylated with 10 mM iodooacetamide in the dark for 20 min at room temperature. Then samples were incubated overnight with MS grade trypsin protease (ratio 1:100, 90057, Thermo Scientific) at 37 °C. The following day, peptides were eluted from the columns and lyophilized. At the end of the lyophilization process, the peptides were suspended in 0.1% formic acid (1002642510, Merck) and diluted to 100 ng/µl before injecting to the LC-MS/MS system (ACQUITY UPLC M-Class coupled to a SYNAPT G2-Si high definition mass spectrometer (Waters)).

2.4. LC-MS/MS analysis and data processing

The liquid chromatography with tandem mass spectrometry analysis and protein identification were performed, similar to previously published protocols [7, 10]. The samples were loaded onto the ACQUITY UPLC M-Class coupled to a SYNAPT G2-Si high definition mass spectrometer (Waters). To equilibrate the columns, 97% of mobile phase (including 0.1% formic acid in UHPLC grade water) was used and column was heated to 55°C. Ninety min gradient elution from the trap column ACQUITY UPLC M-Class Symmetry C18 trap column (180 µm x 20 mm; 186007496, Waters) to the analytic column (ACQUITY UPLC M-Class HSS T3 Column, 100Å, 1.8 µm, 75 µm X 250 mm; 186007474, Waters) at 0.400 µl/min flow rate with a gradient from 4% to 40% hypergrade acetonitrile (100029, Merck) containing 0.1% formic acid (v/v) was used for the peptide separation. Positive ion modes of MS and MS/MS scans with 0.7 sec cycle time were performed sequentially. Ten volts was set as low collision energy and 30 V as high CE. Ion mobility separation (IMS) was used for the ion separation. A wave velocity was ramped from 1000 m/s to 55 m/s over the full IMS cycle. The release time for mobility trapping was set as 500 µs, trap height was set to 15 V. IMS wave delay was 1000 µs for
the mobility separation after trap release [11]. Without any precursor ion preselection, all
the ions within 50-1900 m/z range were fragmented in resolution mode. Additionally, 100
fmol/μl Glu-1-fibrinopeptide B was infused as lockmass reference with a 60 s interval.
Progenesis-QI for proteomics software (Waters) was used for the identification and
quantification of the peptides. Whole proteins were identified by at least 2 unique peptide
sequences and then, expression ratio of proteins was calculated.

Data were further analyzed to understand the role of these proteins in the biological
activities. For this aim, PANTHER software (Protein ANalysis Through Evolutionary
Relationships, http://pantherdb.org) was used and data were classified according to their
role in biological or physiological activities.

2.6. Western blot

Western blotting was carried out as previously described [12]. Briefly harvested tissue
samples from striatum belonging to the same group were pooled, homogenized and
sonicated. Then, samples were treated with Protease/Phosphatase inhibitor cocktail
(5872, Cell Signaling). Total protein concentration was determined via Qubit 3.0
Fluorometer (Q33216, Invitrogen) according to the manufacturer’s protocol. Twenty
microgram protein for each sample were loaded to the 4–20% Mini-PROTEAN TGX
Stain-Free protein gels (4568095, Biorad). Therefore, electrophoresis was conducted at
150 V at 1.5 h for size-fractionation of the proteins. At the end of the gel electrophoresis,
proteins were transferred to a Polyvinylidene fluoride (PVDF) membrane using the
Trans-Blot Turbo Transfer System (1704155, Bio-Rad). Thereafter, membranes were
blocked in 5% non-fat dry milk (Blotto; sc-2324, Santa Cruz Biotechnology) in 50 mMol
Tris-buffered saline (TBS) containing 0.1% Tween (TBS-T; blocking solution) for 1 h at room temperature. After blocking step membranes were washed using 50 mMol TBS-T, and incubated overnight with monoclonal mouse PDE10A (sc-515023; Santa Cruz Biotechnology). On the next day, membranes were washed with 50 mM TBS-T and incubated with horseradish peroxidase-conjugated goat anti-mouse (405306; Biolegend) antibody (diluted 1:5000) for 1 h at room temperature. Blots were performed at least three times. Protein loading was controlled by stripping and re-probing with polyclonal rabbit anti-β-actin antibody (622102; Biolegend). Blots were developed using Amersham ECL Western Blotting Detection Reagents (RPN2209, GE Healthcare) and visualized using the ChemiDoc MP System (1708280, Bio-Rad; Life Sciences Research). Densitometric analysis was performed to analyze protein levels via an image analysis software (Image J; National Institute of Health, Bethesda, MD, USA). Protein levels were corrected with β-actin blots and expressed as relative values compared with control (ZT0) group.

2.7. Statistics

For statistical data comparisons, a standard software package (SPSS 18 for Windows; SPSS Inc., Chicago, IL, USA) was used. Differences between groups were analyzed by independent samples t-test. All values are given as mean ± S.D. with n values, indicating the number of samples or animals analyzed. LC-MS/MS data quantified in Progenesis-QI software was evaluated by independent samples t-test and 1.4-fold change was considered as significant between ZT0 (morning group) and ZT18 (midnight group). Throughout the study, p values < 0.05 were considered significant.
3. Results

To identification of light and dark regulated proteins, tissue samples at two different Zeitgeber time points (ZT0 and ZT18) were taken from mice striatum under physiological conditions. Afterward, samples were analyzed using the LC-MS/MS method and results showed that A-kinase anchor protein 10 (AKAP10), Fructose-bisphosphate aldolase C (ALDOC), Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (AT2A2), Plasma membrane calcium-transporting ATPase 1 (AT2B1), Tyrosine-protein kinase Blk (BLK), Copine-5 (CPNE5), cAMP-dependent protein kinase type II-beta regulatory subunit (KAP3), NADP-dependent malic enzyme (MAON), Neurocalcin-delta (NCALD), Neurofilament light polypeptide (NFL), Nucleophosmin (NPM), cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A (PDE10A), Isoform 5 of Phosphatidylinositol-binding clathrin assembly protein (PICAL), Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma (PI51C), Protein phosphatase 1 regulatory subunit 1B (PPR1B), Proteasome subunit beta type-6 (PSMB6), 60S ribosomal protein L10 (RL10), Sorting and assembly machinery component 50 (SAM50), SH3 domain-binding glutamic acid-rich-like protein 3 (SH3L3), Synaptojanin-1 (SYNJ1), Mitochondrial import receptor subunit TOM70 (TOM70), Tyrosine 3-monoxygenase (TY3H), Vesicle-associated membrane protein-associated protein (VAPA) proteins were significantly altered between ZT0 and ZT18 (Figure 1).

Under physiological conditions AKAP10, ALDOC, BLK, NCALD, NFL, PDE10A, PICAL, PSMB6, RL10, SH3L3 and SYNJ1 protein expressions were significantly altered with respect to dark cycle (Figure 2A-K). On the other hand, AT2A2, AT2B1, CPNE5, KAP3, MAON, NPM, PI51C, PPR1B, SAM50, TOM70, TY3H, and VAPA protein
expressions significantly diminished in the midnight group compared to the morning group (Figure 3A-L).

Proteins changing significantly between ZT0 and ZT18 were classified broadly into several categories according to the Protein ANalysis THrough Evolutionary Relationships (PANTHER, http://pantherdb.org) classification system. According to the molecular function-based classification: six binding (ATP2B1, BLK, CPNE5, NPM1, PICALM, SYNJ1), nine catalytic activity (ALDOC, ATP2A2, ATP2B1, BLK, MAON, PDE10A, PSMB6, SYNJ1, TY3H), two structural molecular activity (ATP2A2, ATP2B1), and one transporter activity (RL10) (Figure 4A). Furthermore, pathway analysis was performed according to the proteomic data (Figure 4B). The top scored pathways were dopamine receptor mediated signaling (KAP3, PPR1B, TY3H), Parkinson’s disease (BLK, TY3H), and nicotine pharmacodynamics (PPR1B, TY3H). In addition, protein class analysis was performed according to the proteomic data. these proteins were mapped to three major protein class: membrane trafficking protein (PICALM, VAPA), metabolite interconversion enzyme (MAON, PIP51C, SYNJ1), and transporter (ATP2A2, ATP2B1) (Figure 4C). Moreover, these proteins were mapped to five major biological process; biogenesis (BLK, NPM1, PICALM, RL10, SYNJ1, VAPA), biological regulation (ATP2A2, ATP2B1, BLK, PDE10A, PPR1B), cellular process (ALDOC, ATP2A2, ATP2B1, BLK, CPNE5, MAON, NPM1, PDE10A, PICALM, PPR1B, PSMB6, RL10, SYNJ1, TY3H, VAPA) localization (ATP2A2, BLK, PICALM, SYNJ1, VAPA) and metabolic process (ALDOC, BLK, MAON, PSMB6, SYNJ1, TY3H) (Figure 4D).
To validate and confirm proteomics data, PDE10A protein expression was analyzed from brain tissue via Western blot (Figure 5A). As in proteomics data, PDE10A protein level significantly increased in the ZT18 group compared to the ZT0 group under physiological conditions.

4. Discussion

The suprachiasmatic nucleus (SCN) of the hypothalamus known as the master circadian clock in mammals, regulates the clock for the body's circadian rhythms [13]. It is well known that circadian rhythm regulates most of our physiological functions including the sleep-wake cycle, endocrine system, heart rate, and energy metabolisms [1, 2]. Recent studies suggested that circadian rhythm plays an important role in the pathophysiology of neurodegenerative diseases such as cerebral ischemia, Alzheimer's disease and Parkinson's disease [13-15]. Furthermore, it is speculated that the clock mechanisms have a direct or indirect effect on both the triggering of these neurological diseases and the subsequent damage mechanisms.

In our previous study, we showed that mice with night-time focal cerebral ischemia could better tolerate ischemic injury in mice. In addition, in the same report, we also analyzed protein expression profiles in different time points (ZT0, ZT6, ZT12, and ZT18) using proteomic analyses. We showed that calcium/calmodulin-dependent serine protein kinase (CSKP), guanine nucleotide-binding protein z subunit alpha (GNAZ), neuronal growth regulator 1 (NEGR1), imprinted and ancient protein (IMPACT), calcium/calmodulin dependent 3',5'-cyclic nucleotide phosphodiesterase 1B (PDE1B), and hemoglobin
subunit beta-1, beta-2, and alpha (HBB1, HBB2, HBA) protein expressions were significantly altered between morning and midnight in the ischemic brain tissue [7].

Our main aim in this study was to identify the light and dark regulated proteins in the mouse brain via LC-MS/MS, which is the most appropriate and popular technique in proteomic analyses to identify proteins. Here, we identified 1621 different proteins between ZT0 and ZT18 group. Apart from the core clock proteins (BMAL1, CLOCK, PER1 and PER2), 23 proteins were differentially expressed, 12 downregulated (AT2A2, AT2B1, CPNE5, KAP3, MAON, NPM, PI51C, PPR1B, SAM50, TOM70, TY3H and VAPA), and 11 upregulated (AKAP10, ALDOC, BLK, NALD, NFL, PDE10A, PICAL, PSMB6, RL10, SH3L3 and SYNJ1), in ZT18 group as compared with ZT0 group.

Among these, four proteins (AKAP-10, KAP3, PDE10A and PPR1B) are directly or indirectly associated with the cyclic adenosine monophosphate (c-AMP) signaling pathway. AKAP-10 binds to the regulatory subunit of the cAMP-dependent protein kinase also known as protein kinase A (PKA) which is a serine/threonine protein kinase [16]. Both KAP3 and PDE10A take part in cyclic-AMP signaling. is highly enriched in medium spiny neurons (MSNs) of the mammalian striatum and it is involved in dopamine signaling [17, 18]. Recent studies demonstrated that PDE10A inhibition contributed to activation of D1-direct and D2-indirect pathway and also its inhibition by orally activated TAK-063 reduces brain infarct volume after cerebral ischemia [19, 20]. PPR1B also known as dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32,000 (DARP-32) which is a potent inhibitor of protein phosphatase-1 [21]. It was suggested that DARP-32 which is highly abundant neuros regulates many physiological
functions including ion channel permeability and synaptic plasticity [22]. Although AKAP10 and PDE10A protein expressions are high at midnight, KAP3 and PPR1B protein levels are high in the morning hours under physiological conditions.

NCALD, AT2A2 (also known as SERCA2) and AT2B1 are play pivotal role in the calcium homeostasis. NCALD primarily involved in neuronal calcium signaling mechanism and it is speculated that there is a close relationship between NCAL and adult neurogenesis [23]. SERCA2 is an intracellular calcium pump on the endoplasmic or sarcoplasmic reticulum, plays an essential role in regulating calcium homeostasis [24, 25]. Like SERCA2, AB2B1 is also responsible for calcium transportation. Although NCALD protein expression is high at the midnight, SERCA2 and AT2B1 protein expressions are high in the morning.

Results obtained from this study showed that ALDOC, BLK, NFL, PICAL, PSMB6, RL10, SH3L3 and SYNJ1 protein expressions are higher at midnight compared to the morning. It is reported that cardiovascular diseases or neurological disorders increase ALDOC protein expression which plays an essential role for the repair of injured brain tissue [26]. BLK is also known as B lymphoid tyrosine kinase belonging to the SRC family kinases. It is responsible for development of B-cell and involved in B-cell receptor signaling [27, 28]. In addition, it is predicted that SRC is a non-receptor tyrosine kinase that plays an important role in the Parkinson’s disease which is the second most common neurodegenerative disorders after Alzheimer’s disease [29]. PSMB6 also known as 20S proteasome subunit beta-1 which codes for the β1 core catalytic subunit of the proteasome [30]. RL10 is one of large ribosomal proteins and plays an important role in cell
proliferation, migration and differentiation [31]. The function of SH3L3 is largely unknown but it was suggested that it is upregulated in many cancer types including pancreatic and lung [32]. Furthermore NFL, PICAL and SYNJ1 proteins play an essential role in Alzheimer’s disease [33-35]. NFL also known as neurofilament light chain, is a biomarker for determining axonal degeneration. Studies showed that increasing blood NFL level indicates the severity of the neurodegenerative diseases including Alzheimer’s disease [33]. SYNJ1 is highly expressed in nerve terminals and it is important for vesicle trafficking and coating/uncoating of endocytic vesicles [35, 36]. In addition, it is speculated that SYNJ1 may essential target for Alzheimer’s disease.

In addition, CPNE5, MAON, NPM, PI51C, SAM50, TOM70, TY3H and VAPA protein expressions were significantly decreased at midnight group compared to the morning group under physiological conditions. CPNE5 is highly expressed during embryonic development and it is speculated that it may play a pivotal role for the development of mouse brain [37]. In a study using a CPNE5 knockout mice, CPNE5 deficiency reduce the anxiety level in rodents [38]. Mitochondrial MAON is encoded by human ME3 gene, is a pivotal member of malic enzyme family. It plays essential roles in normal insulin secretion and promotes epithelial-mesenchymal transition in pancreatic cancer cells [39, 40]. B23, also known as nucleophosmin (NPM) is an neuronal survival factor which interacts with survival kinase Akt [41]. Type I Phosphotidylinosotol 4-Phosphate 5-Kinase (PIP5KI) has three isoforms, α, β, and γ. The PIP5KI γ highly abundant in brain and plays important role in cytoskeletal organization [42, 43]. SAM50 and TOM70, are critical regulators for mitochondria, decreases in the midnight period under physiological conditions. SAM50 is a vital component of the sorting and assembly machinery (SAM)
which is an important controller of mitochondrial dynamics [44]. In addition, TOM70 is
the essential subunit of the translocase of the outer membrane (TOM) which is facilitating
the import of mitochondrial proteins from the cytosol [45]. TY3H also known as tyrosine
hydroxylase responsible for catalyzing the initial and rate-limiting step in the biosynthetic
pathway of catecholamines (dopamine, noradrenaline, and adrenaline), thereby directly
linking Parkinson’s disease with tyrosine hydroxylase [46-48]. VAPA is participates in
cell movement, protein complex assembly and vesicular transmission [49].

Classification of identified proteins depending on molecular function demonstrated that
significantly altered proteins interacted with binding, catalytic activity, structural
molecule activity and transporter activity related proteins. Therefore, three main signal
transduction pathways were identified as dopamine receptor mediated signaling,
Parkinson’s disease, and nicotine pharmacodynamics. In addition to this, PANTHER
classification based on protein class included 3 predominant groups: membrane
trafficking protein, metabolite interconversion enzyme, and transporter. Moreover, these
proteins were mapped to five major biological process, biogenesis, biological regulation,
cellular process, localization and metabolic process.

Recently, protein identification from brain tissue via LC-MS/MS is the most appropriate
and popular techniques. However, in order to increase the reliability of proteomic data,
confirmation was made by Western blot. For this purpose, PDE10A protein expression
was analyzed and results supports the proteomic data.
Taken altogether, our findings demonstrated that AKAP10, ALDOC, AT2A2, AT2B1, BLK, CPNE5, KAP3, MAON, NCALD, NFL, NPM, PDE10A, PICAL, PI51C, PPR1B, PSMB6, RL10, SAM50, SH3L3, SYNJ1, TOM70, TY3H, and VAPA proteins are regulated by light and dark cycle in mouse brain. Therefore, these proteins might have an impact on the circadian rhythm. Further studies are needed to clarify the role of these proteins on the regulation of circadian rhythm.

Acknowledgement/Disclaimers/Conflict of interest:

None.
References


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**Figure 1.** List of differentially expressed proteins with respect to ZT0 and ZT18 mice via liquid chromatography mass spectroscopy (LC-MS/MS). A total of 24 different proteins were significantly regulated (p < 0.05 and >1.4-fold change between ZT0 and ZT18) depending on the light and dark period.
Figure 2. Significantly increased proteins compared to the ZT0 (morning group) via liquid chromatography mass spectroscopy (LC-MS/MS). (A) A-kinase anchor protein 10 (AKAP10), (B) Fructose-bisphosphate aldolase C (ALDOC), (C) Tyrosine-protein kinase Blk (BLK), (D) Neurocalcin-delta (NCALD), (E) Neurofilament light polypeptide (NFL), (F) PDE10A, (G) PICAL, (H) PSMB6, (I) RL10, (J) SH3L3, (K) SYNJ1.
(F) cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A (PDE10A), (G) Isoform 5 of Phosphatidylinositol-binding clathrin assembly protein (PICAL), (H) Proteasome subunit beta type-6 (PSMB6), (I) 60S ribosomal protein L10 (RL10), (J) SH3 domain-binding glutamic acid-rich-like protein 3 (SH3L3), and (K) Synaptojanin-1 (SYNJ1). All data are expressed as means ± S.D. values. **p<0.01/ *p<0.05 compared with ZT0 group.
Figure 3. Significantly decreased proteins compared to the ZT0 (morning group) via liquid chromatography mass spectroscopy (LC-MS/MS). (A) Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (AT2A2), (B) Plasma membrane calcium-transporting ATPase 1 (AT2B1), (C) Copine-5 (CPNE5), (D) cAMP-dependent protein kinase type II-beta regulatory subunit (KAP3), (E) NADP-dependent malic enzyme (MAON), (F)
Nucleophosmin (NPM), (G) Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma (PI51C), (H) Protein phosphatase 1 regulatory subunit 1B (PPR1B), (I) Sorting and assembly machinery component 50 (SAM50), (J) Mitochondrial import receptor subunit TOM70 (TOM70), (K) Tyrosine 3-monooxygenase (TY3H), and (L) Vesicle-associated membrane protein-associated protein A (VAPA). All data are expressed as means ± S.D. values. **p<0.01/ *p<0.05 compared with ZT0 group.
Figure 4. Classification of light and dark regulated proteins in mice striatum. Circadian rhythm related proteins were classified with respect to (A) molecular function, (B) pathway, (C) protein class, and (D) biological process via Protein ANalysis Through Evolutionary Relationships (PANTHER, http://pantherdb.org) classification system.
Figure 5. PDE10A protein expression (A) was analyzed by Western blot to confirm proteomic analysis. Representative images of Western blot analysis were given above their corresponding graphs. All data are expressed as means ± SD values (n = 3 blots/protein). **p<0.01/ *p < 0.05 compared with ZT0 group.