Possible Role of Autophagy in Monosodium Glutamate-induced Bladder Overactivity in Adult Male Rats

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Abstract:

Introduction

Autophagy is an intracellular recycling process that protects cells against various cellular stressors, including oxidative stress, through autophagosome formation and degradation. LC3-II and Beclin-1 are regarded as the key proteins responsible for autophagosome formation. Overactive bladder (OAB) is a bladder disorder characterized by increased sensitivity of the detrusor muscle, leading to urgency, and a common contributor underpinning the pathophysiology of the condition is increased production of local ROS. Hence, we investigated the role of autophagy as a possible mechanism for improving machinery in the MSG MSG-induced OAB rat model.

Patients and Methods

Two groups of adult male Wister albino rats (n=10) were kept for 4 weeks and randomly designated into a control group that received the vehicle, 0.9% saline orally, and a monosodium glutamate (MSG) group that received saline dissolved monosodium glutamate orally for induction of bladder overactivity. Following sacrifice, assessment of 10-minute induced acetylcholine (ACh) detrusor muscle contractile activity of the study groups in vitro was recorded. Measurement of malondialdehyde (MDA) level in bladder tissue homogenate was conducted by ELIZA; meanwhile, gene expression of detrusor muscle LC3-II and Beclin-1 was measured by qPCR.

Results

A significant increase in the 10-minute ACh-induced bladder contraction of the MSG group was associated with significantly higher MDA levels and upregulation of LC3-II and Beclin-1 mRNA expression compared to the control group.

Conclusion

Autophagic machinery is highly activated in OAB conditions due to increased local ROS production, but unfortunately, it didn't improve the condition.

Abbreviations

OAB; Overactive bladder, MSG; monosodium glutamate, ROS; Reactive oxygen species, MDA; malondialdehyde, LC3-II; microtubule-associated protein-light chain 3, qPCR; quantitative polymerase chain reaction, ULK; Unc-51-like kinase, Atg; Autophagy-related protein, FIP200; the focal adhesion kinase family interacting protein of 200 kD, Vps34; Vacuolar protein sorting 34, PI3K CIII; Phosphatidylinositol 3-Kinase class III.

Keywords

Overactive bladder, monosodium glutamate, Beclin-1, MDA.

Introduction:

Autophagy, a term derived from the Greek words "auto" meaning "self" and "phagy" meaning "eating," is a fundamental cellular process that degrades and recycles intracellular components, especially the misfolded proteins and damaged organelles, to maintain homeostasis (1, 2). A multitude of cellular stressors, such as nutrient starvation, inflammation, and organelle damage, highly induce this reparative process (3).

The process of autophagy involves three sequential steps starting with the formation of the phagophore, followed by elongation and engulfment of the damaged structure forming an autophagosome, and lastly degradation through fusion with a lysosome, forming an autolysosome for reusing the resulting macromolecules (4, 5). Two essential complexes are involved in the initial formation of the phagophore: the ULK1 complex, including (ULK1/2, Atg13, FIP200), and the PI3K CIII complex composed of (Vps34, Vps15, Atg14, Beclin-1). Meanwhile, the process of membrane elongation for engulfment requires two sequential steps of ubiquitination-like reaction. The first ubiquitination reaction is achieved by the ATG conjugation system, followed by the covalent conjugation of phosphatidylethanolamine (PE) to microtubule-associated protein-light chain 3 (LC3) to facilitate membrane expansion and closure (6).

According to the International Continence Society, overactive bladder (OAB) is a symptomatic health condition characterized by abnormal urinary urgency often accompanied by voiding frequency in the absence of any obvious urinary tract infections and/or other underlying pathologies (7). Its worldwide prevalence ranges from 11.8% to 35.6% (8), with higher incidence in women and with aging (9).

It is believed that the major contributors to the underlying pathophysiological changes of the condition are local inflammation and increased production of local reactive oxygen species (ROS) (10, 11). Both can alter the urothelium's sensory and barrier functions, increasing the sensory nerves' sensitivity and detrusor muscle responsiveness (12, 13).

Recent studies have elucidated the intricate mutual relationship between ROS and autophagy. Redox signaling, primarily mediated by ROS, plays a pivotal role in regulating autophagic flux through activation of different autophagy-related pathways (1, 14) such as AMP-Activated Protein Kinase (AMPK) Pathway (15), p62/Nrf2 Pathway (1), Hypoxia-Inducible Factor-1 (HIF-1) (16), and lastly Forkhead Box O (FOXO) Transcription Factors to upregulate the expression of autophagy proteins; LC3-II and Beclin-1 (17).

Conversely, autophagy modulates ROS levels through several mechanisms, including degradation of oxidized proteins, removal of ubiquitinated protein aggregates, and elimination of damaged mitochondria, a major source of ROS production within cells (1, 18). This bidirectional regulation between ROS and autophagy underscores their possible critical roles in the pathogenesis of OAB. Hence, the present study aimed to investigate the possible role of autophagy as an ameliorative or a collaborative process in the pathogenesis of bladder overactivity.

Materials and Methods

I. Animal groupings and experimental design:

Twenty adult male Wistar Albino rats aged 12 weeks, weighing 150–200 g, purchased from the Animal House of the Faculty of Veterinary Medicine, Assiut University, were assigned for the study. After one week of acclimatization under a natural light–dark cycle, with free access to chow diet and *ad libitum*, rats were randomly allocated to two groups (n =10) as the **Control group:** received 1 ml of the vehicle orally (0.9% saline) daily (19). **Monosodium glutamate group (MSG):** received 5g/kg/day monosodium glutamate dissolved in 1 ml of 0.9% saline orally (20, 21) for induction of bladder overactivity for 4 weeks. The experimental procedures were

carried out according to the Guidelines for the Care and Use of Laboratory Animals.

They were approved by the Ethical Committee IRB no: 17101442 at the Faculty of Medicine, Assiut University, Egypt.

II. Tissue sampling:

Urinary bladder dissection:

Following decapitation, a cautious longitudinal cut of the lower abdomen, followed by the bladder, was performed in a manner to form a flat sheet (22). Subsequently, the sheet was cut longitudinally into three parts; the first was used to evaluate bladder contractile activity. For biochemical analysis, homogenization of the second part in PBS (0.01 M, pH 7.4) was performed, and the last part was planned for qPCR assessment.

A. Evaluation of urinary bladder contractile activity:

The bladder strip was kept at 37 °C, continuously bubbled with carbogen, and equilibrated for 60 minutes while suspended in the freshly made Krebs' solution (Automatic Isolated Organ Bath System-Orchid Scientific No: OB-1D: India). A force transducer (Power Lab 26T, Australia) was connected to the preparation, and the isometric tension was measured. A resting tension of 1 g was kept constant throughout the procedure to calibrate the record. The muscle's reaction to acetylcholine (Acros Organics, USA) at a 10-3M concentration after 10 minutes of instillation was evaluated. (19, 23).

B. Assessment of Malondialdehyde (MDA) in the homogenate of the urinary bladder with ELIZA:

The supernatant collected after homogenization was analysed via the commercially available ELIZA kits for MDA in nM/L *(CAT. No. MD 25 29, Biodiagnostic, Egypt)* according to the manufacturers' protocols (19).

C. Determination of tissue LC3-II and Beclin-1 gene expression using Quantitative PCR:

The QIAamp RNA Mini Kit (Qiagen, USA) extracted total RNA from bladder tissue samples. Then, in accordance with the manufacturer's instructions, a High-Capacity cDNA synthesis kit (Thermo Fisher Scientific Co., Fremont, California, USA) was used to reverse-transcribe the RNA. Subsequently, A Biosystem 7300 (Applied Biosystems, CA, USA) qPCR detection system and a QuantiTect SYBR Green PCR Kit (Qiagen, USA) were employed to perform a SYBR Green-based quantitative polymerase reaction (qPCR).

The following sequences for LC3-II, Beclin-1 (24, 25), and GAPDH (as an internal housekeeping control) primers (25, 26) were used as shown in **Table 1.**

Table 1: The primer sequences for LC3-II, Beclin-1, M3, Cx43, and GAPDH.

Primer	**Sequence (5' - 3')**
LC3-II	F: CATGGGCACAGATGAAGACAC R: GCCAGATGTTCATCCACTTTC
Beclin-1	F: ACCAGGAGGAAGCTCAGTACC R: CAGGCAGCATTGATTTCATTC
GAPDH	F: ATGGGAGTTGCTGTTGAAGTCA R: CCGAGGGCCCACTAAAGG

III. Statistical Analysis

Data were analysed using SPSS version 20 (SPSS Inc., Chicago, USA). After testing data for normality (Shapiro-Wilk test for normality), the Data were normally distributed and analysed using Student's t-test. Then the results were presented as mean ± standard deviation (SD) and considered significant if the *P* value ≤ 0.05. Next, Pearson's correlation analysis test was performed.

IV. Funding

The study was funded by the Grant Office, Faculty of Medicine, Assiut University (fund no. 2021-03-29-005).

Results

I. Urinary bladder contractile activity in response to acetylcholine:

After 10 minutes of acetylcholine administration, the MSG group showed a significant increase in contractile force compared to the control group, as shown in **Table 2 and Figure 1.**

Table 2: Detrusor muscle contractile activity after 10 minutes of acetylcholine (ACh) administration in the studied groups with a resting maintained tension of 1 g throughout the experiment.

Parameter / **Groups**	**Control**	**MSG**
Urinary Bladder Contractility (g tension)	2.42 ± 0.14	4.08 ± 0.55*****

Data are represented as means ± SD (n = 10 in each group). MSG (Monosodium glutamate group). *****: statistically significant difference compared to Control (*p* value < 0.05), Student's t-test was used for analysis.

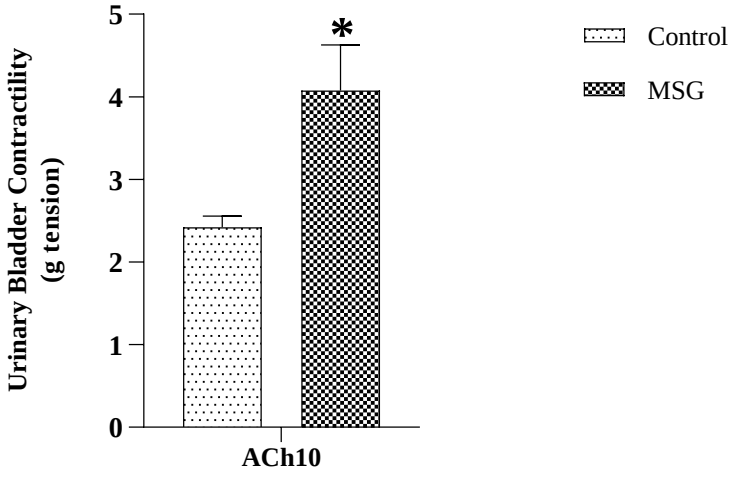

Figure 1: Detrusor muscle contractile activity (10 mins of ACh administration) in the studied groups in gram tension units. Data are represented as means ± SD (n = 10 in each group). MSG (Monosodium glutamate group), ACh (acetylcholine), *****: statistically significant difference compared to Control (*p* value < 0.05), Student's t-test was used.

I. <u>Urinary bladder malondialdehyde (MDA) level:</u>

Bladder tissue of the MSG group exhibited a significant increase in MDA levels compared to the control group (9.10 ± 0.32 nM/L vs. 8.12 ± 0.44 nmol/L; *p* value < 0.05), as shown in **Figure 2.**

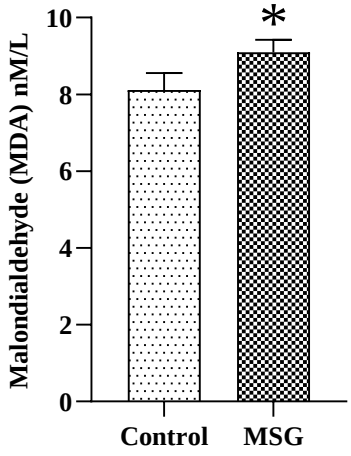

Figure 2: Urinary bladder malondialdehyde (MDA) in the studied groups. Data are represented as means ± SD (n = 10 in each group). MSG (Monosodium glutamate group), MDA (Malondialdehyde). *****: statistically significant difference compared to Control (*p* value < 0.05). Student's t-test was used for analysis.

II. <u>Quantitative – PCR results</u>

A. Urinary bladder LC3-II expression:

Monosodium glutamate administration in the MSG group caused a significant increase in the expression of LC3-II in the detrusor muscle compared to the control group (*p* value < 0.05), as shown in Figure 3, panel A.

B. Urinary bladder Beclin-1 expression:

Similarly, monosodium glutamate administration in the MSG group caused a significant increase in the expression of Beclin-1 in the detrusor muscle compared to the control group (*p* value < 0.05), as shown in **Figure 3, panel B.**

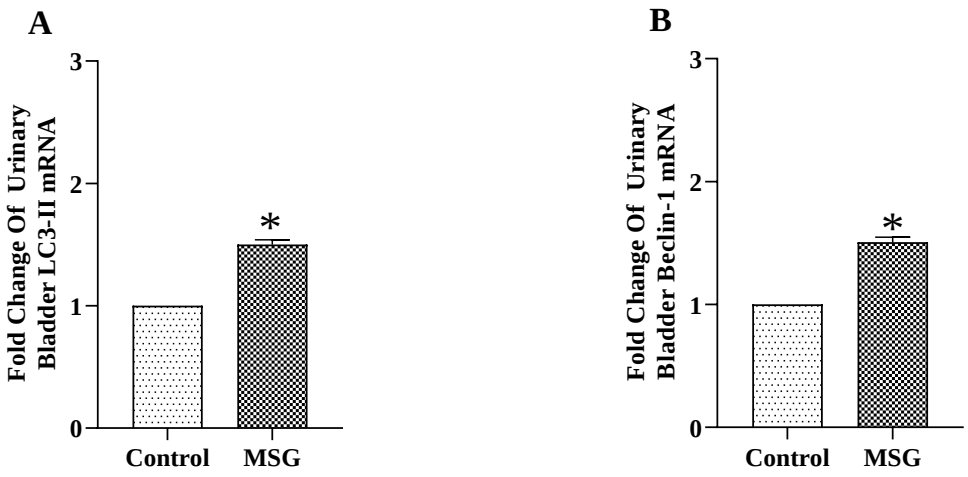

Figure 3: Urinary bladder expression of autophagy markers (panel A; fold change of bladder LC3-II mRNA) and (panel B; fold change of bladder Beclin-1 mRNA). Data are represented as means ± SD (n = 10 in each group). MSG (Monosodium glutamate group), *****: statistically significant difference compared to Control (*p* value < 0.05). Student's t-test was used for analysis.

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