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Thioredoxin-1 expression regulated by morphine in SH-SY5Y cells

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HIGHLIGHTS

- Morphine induced thioredoxin-1 expression.
- Morphine did not influence thioredoxin binding protein-2 expression.
- Naloxone inhibited the increased level of thioredoxin-1 caused by morphine.
- Morphine induced thioredoxin-1 expression through PI3K and ERK pathways.

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ABSTRACT

Attempts are being made to identify genes targeted by morphine. It is beneficial for developing new treatments that alleviate side-effects of morphine. Thioredoxin-1 is a small ubiquitous protein that has various biological activities, such as the control of redox balance, the inhibition of apoptosis and the modulation of inflammation. In this study, we found that thioredoxin-1 was induced by morphine in SH-SY5Y cells. Furthermore, opioid receptor, PI3K and ERK pathways were involved in morphine-induced increase of thioredoxin-1 expression. These results suggest that thioredoxin-1 maybe play a role in the actions of morphine. More detailed analysis could clarify cellular and molecular mechanisms involved in the actions of morphine.

1. Introduction

Morphine is the most effective opioid analgesic that is widely used for acute and chronic severe pain. However, repeated administration of morphine leads to various side-effects such as antinociceptive tolerance, hyperalgeisa and drug addiction. Accordingly, the clinical utility of morphine is strictly limited and controlled. The mechanisms by which morphine induced these side-effects remain unclear, although a role for apoptosis, structural plasticity, neuroimmune activation and dysfunctions of neurotransmitter release have all been demonstrated [6,29]. More researches are necessary to determine the exact cellular and molecular mechanisms involved in morphine action.

The cellular redox balance is crucial for the homeostasis of living cells. Recent studies indicated that many pharmacological actions of morphine are associated with cellular redox [35,36]. The levels of reducing molecules such as catalase, superoxide dismutase and glutathione were reduced by morphine treatment [32]. The formation of peroxynitrite plays an important role in the development of morphine antinociceptive tolerance [12]. The administration of antioxidants could decrease the dose of morphine in pain treatment and prevent against morphine-induced side-effects [18,32].

Thioredoxin-1 (Trx-1) is a small ubiquitous cytosolic protein with two redox-active cystine residues in its active site sequence: -Cys-Gly-Pro-Cys-. Trx-1 is induced in response to a variety of stresses, such as X-ray and ultraviolet irradiation, hydrogen peroxide, viral infection and ischemic reperfusion [24]. Trx-1 has cytoprotective effects through scavenging radicals by itself or in cooperation with peroxiredoxin [8,11]. In addition, Trx-1 plays critical roles in the signal transduction mediated by AP-1, NF-κB, P53 and p38 MAP kinase via thiol redox control [20]. Thioredoxin binding protein-2 (TBP-2) serves as a negative regulator of the biological function and expression of Trx-1. The Trx-1-TBP-2 interaction plays an important redox regulatory mechanism in cellular processes, including differentiation of myeloid and macrophage lineages. Considering the important role of Trx-1 in maintaining the cellular reducing environment, this raises a question whether Trx-1 expression is affected by morphine.

2. Materials and methods

2.1. Chemicals

The Trx-1 antibody was obtained from Redox Bioscience (Japan). The TBP-2 antibody was purchased from MBL (Nagoya, Japan). The GAPDH antibody was purchased from Abmart (Shanghai,

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China). The primary antibody against p-Akt (Ser-474), Akt, p-ERK (Tyr-204), ERK and β-actin were supplied by Santa Cruz Biotechnology (USA). Morphine hydrochloride was obtained from Shenyang First Pharmaceutical Factory (Shenyang, China). Naloxone hydrochloride was supplied from Beijing Four Rings Pharmaceutical Technology Co., Ltd (Beijing, China). LY294002 and PD98059 were purchased from Biosource (Camarillo, CA, USA). Nuclear and Cytoplasmic Protein Extraction Kit was obtained from Bioteke Corporation (Beijing, China).

2.2. Cell culture

SH-SY5Y cells and PC12 cells were supplied from Kunming Institute of Zoology (Kunming, China). SH-SY5Y cells were maintained in Dulbecco's modified Eagle's medium with 15% heat-inactivated fetal bovine serum, antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin), 15 mM HEPES and 2 mM glutamine at 37 °C in a humidified 5% CO_2 atmosphere. PC12 cells were maintained in RPMI 1640 medium with 10% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum and antibiotics at 37 °C in a humidified 5% CO_2 atmosphere.

2.3. Western blot

Cells were lysed with the solubilizing solution (20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM PMSF, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na_3VO_4, 1 mM β-glycerolphosphate and 1 mg/mL leupeptin). An equal quantity of proteins was separated by 12% or 15% SDS-PAGE and transferred to a PVDF membrane (Millipore Corporation, Billerica, MA, USA). The membranes were soaked in 10% skim milk (in PBS, pH 7.2, containing 0.1% Tween-20) overnight at 4 °C, then incubated with primary antibodies followed by peroxidase-conjugated anti-mouse or anti-rabbit IgG (KPL, Gaithersburg MD, USA). The epitope was visualized by an ECL Western blot detection kit. Densitometry analysis was performed by using ImageJ software.

2.4. Transfection and luciferase assay

pTrx (-1148)-luciferase and control vectors are provided by Dr. Junji Yodoi and Hiroshi Masutani. SH-SY5Y cells were transfected with luciferase reporter expression vectors according to the manufacturer's instructions. After 48 h, transfected cells were treated with 10 μM morphine. For controlling the efficiency of transfection, *Renilla* luciferase gene expression was monitored using pRL-TK. The assay was performed with the luciferase gene expression assay kit (Promega).

2.5. Data analysis

Data were expressed as means ± SD values. Statistical analysis was performed by using SPSS software. The one-way ANOVA

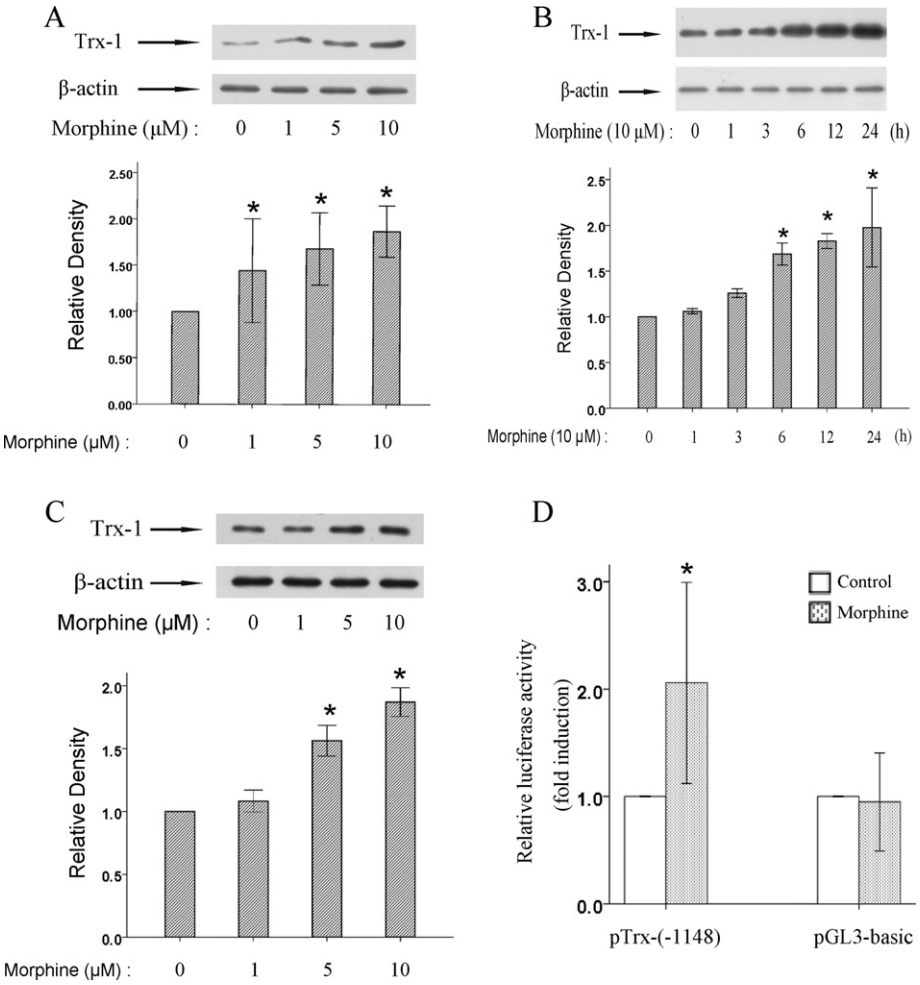

Fig. 1. Trx-1 was induced by morphine in SH-SY5Y cells. (A) SH-SY5Y cells were treated by morphine (1, 5, and 10 μM) for 24 h, and then Trx-1 expression was detected by Western blot. (B) SH-SY5Y cells were treated by 10 μM morphine for 1, 3, 6, 12 and 24 h, and then Trx-1 expression was detected by Western blot. (C) PC12 cells were treated by morphine (1, 5, and 10 μM) for 24 h, and then Trx-1 expression was detected by Western blot. (D) SH-SY5Y cells transfected with pTrx (-1148)-luciferase vector were treated with morphine (10 μM) for 24 h. The data quantified by Image J software were shown in the down panel. Asterisks indicate statistical significance (*$P < 0.05$).