Department of Biochemistry and Microbiology

Morphine Potentiates Glucocorticoid Receptor Translocation in Neuronal Cells.

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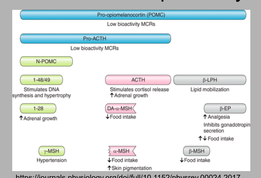
INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in regulating signaling by glucocorticoid receptor which is expressed in almost all cells. Adrenocorticotropin hormone (ACTH) and β-endorphin both of which are derived through processing of pro-opiomelanocortin (POMC) pro-hormone are secreted from anterior pituitary under stressed conditions. ACTH released into circulation regulates the release of glucocorticoids from adrenal gland. Glucocorticoids cause profound suppression of functional activity of HPA axis as negative feedback control. The endogenous opioids acting primarily at mu opioid receptor inhibit activity of HPA axis and thus release ACTH and β-endorphin from anterior pituitary. Furthermore, there are enough reports to support that glucocorticoids regulate mu opioid receptor expression through GRE binding specially in mouse where it has already been shown that promoter region of mouse mu opioid receptor not delta or kappa opioid receptor contains a glucocorticoid-response element (GRE). The glucocorticoid receptor is a member of steroid-hormone-receptor family of proteins. It binds to glucocorticoids with high affinity. In inactive state, the GR complexes with chaperones like heat shock proteins 70 (Hsp70) and 90 (Hsp90) and immunophilins and their co-chaperones making GR more accessible to ligand binding. After ligand binding, the GR is activated and chaperones and co-chaperones are reshuffled with GR to be translocated to nucleus where the GR homodimerize and binds to GRE in promoter region. The resulting complex recruits either co-activator or co-repressor proteins that modify the structure of chromatin thereby facilitating or inhibiting assembly of the basal transcription machinery and the initiation of transcription by RNA polymerase II.



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METHODS

A murine neuroblastoma cell line, Neuro2A was used in this study. In some experiments, Neuro2A stable cell line was established expressing either mu opioid receptor, Hsp70 or both.

- Cells were treated with morphine sulfate (MS, 1μM) for 5hrs followed by treatment with different concentrations of corticosterone for 30 minutes.
- Following treatments, the cells were collected and protein/RNA were extracted.
- Nuclear fractions (NF) and cytosolic fractions (CF) were extracted to separate nuclear proteins and cytosolic proteins.
- For pull-down experiments, respective antibodies were used to immunoprecipitated the interacting proteins.
- For confocal images of GR translocation experiments, immunocytochemistry was used to fluorescently tag GR protein.

RESULTS

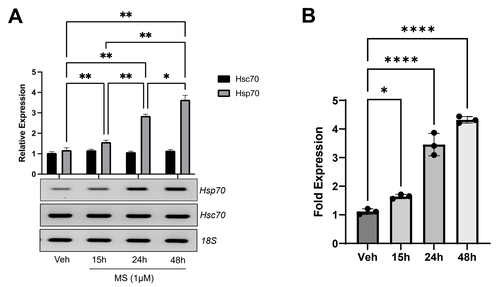


Fig. 1: Morphine treatment up-regulates the message level of Hsp70 in neuronal cells. Neuro2A cells were treated with morphine for indicated time and extracted RNA/cDNA was used to measure Hsp70/Hsc70 message level (**A**) and fold expression using real-time PCR (**B**). 18S was used as an internal control. There is a time dependent increase in message level of Hsp70.

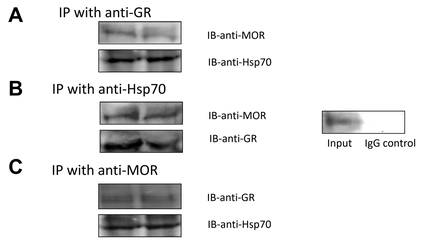


Fig. 2: Immuno-precipitation assay: Total cell lysate of Neuro2A cells stably transfected with HA tagged mu opioid receptor was used to pull down GR, MOR and Hsp70 using antibodies against **A)** GR, **B)** MOR **C)** Hsp70.

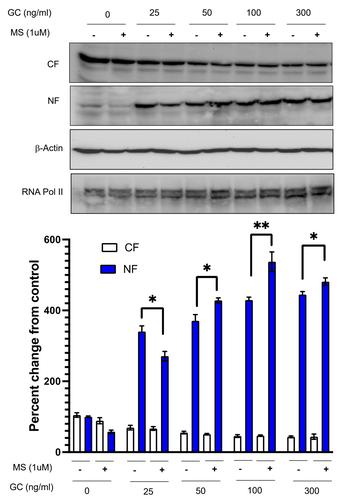


Fig. 3: Morphine treatment potentiates the GR translocation to nucleus: NMT cells were treated with morphine followed by treatment with different concentration of corticosterone. Fifty micrograms of cytosolic (CF) and nuclear fraction (NF) proteins were separated. The bands were expressed as percent of control treatment. β-actin and RNA polymerase II was used as loading control for CF and NF respectively.

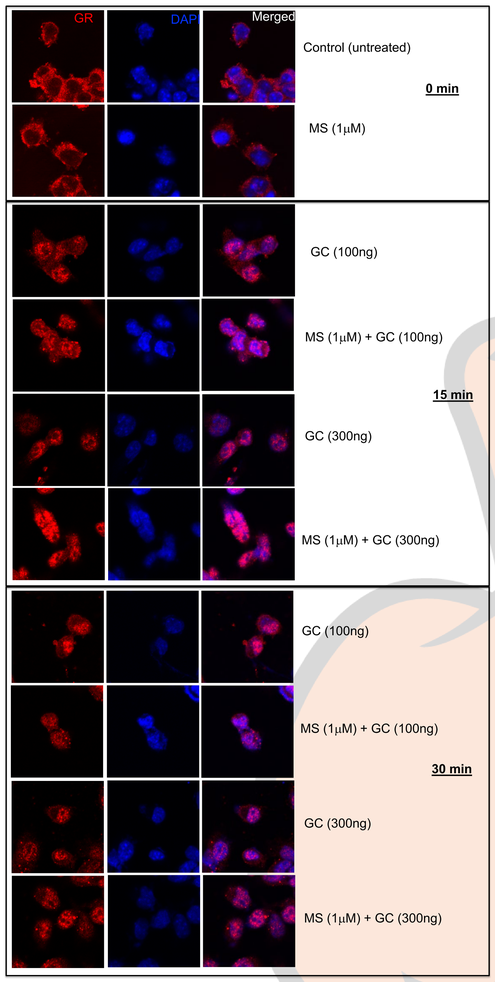


Fig. 4: Confocal imaging of GR translocation: Fixed treated/control Neuro2A cells were stained with mouse anti-GR. The secondary antibody used was goat anti-mouse conjugated with Alexa-Fluor-555. The nucleus was visualized using DAPI. (Magnification = 200x)

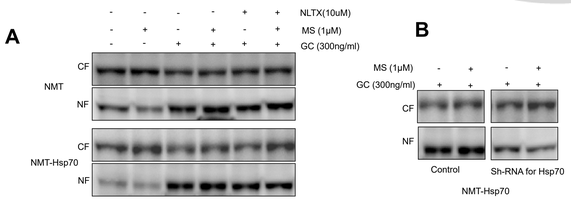


Fig. 5: Hsp70 over-expression potentiates the morphine effect in neuronal cells: Naltrexone (NLTX) treatment inhibited the MS-induced potentiation suggesting classical opioid pathway **(A)**. Knocking-down Hsp70 expression resulted in in decreased GR translocation suggesting this translocation was mediated by Hsp70. The bands were expressed as percent of control treatment.

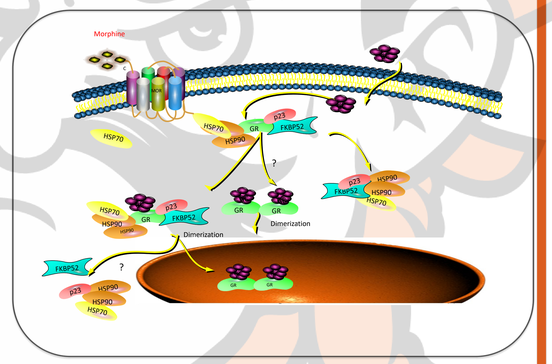
CONCLUSION

Morphine potentiates GR translocation in neuronal cells.

- Morphine treatment increases Hsp70 expression in neuronal cells resulting in time-dependent increase in message levels of Hsp70.
- The pull-down experiments showed that proteins Hsp70, mu opioid receptor and glucocorticoid receptor do interact together.
- Morphine treatment potentiates GR translocation in neuronal cells.
- Over-expression of Hsp70 facilitates GR nuclear translocation
- Silencing of Hsp70 reverses the morphine-mediated potentiation
- Morphine modulation of nuclear translocation of GR involves classical opioid receptor pathway

FUTURE DIRECTIONS

- We have early results suggesting morphine inhibits GR translocation in immune cells and macrophages.
- We would like to confirm the role of Hsp70 in morphine-induced inhibition in immune cells.
- Further confirm the downstream signaling pathways resulting in morphine-induced GR translocation potentiation.



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