TOTAL PROTEIN EXTRACTION FROM MICE HIPPOCAMPI AND PC12 CELLS

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/ CONTEXT

Glucocorticoid hormones are released during the active phase of the circadian cycle and in response to stress. Stress-induced high levels of glucocorticoids have been shown to increase the memory of stress-associated events and to contribute to the development of stressrelated pathologies (depression, anxiety, drug abuse...). This study provides a complete molecular pathway (GR/Egr-1/MAPK/Synapsin-Ia-Ib) through which stress and glucocorticoids enhance the memory of stressrelated events. The study involved total protein extractions both from mice hippocampi and PC12 cells using Precellys®24 homogenizer [1].

/ MATERIALS

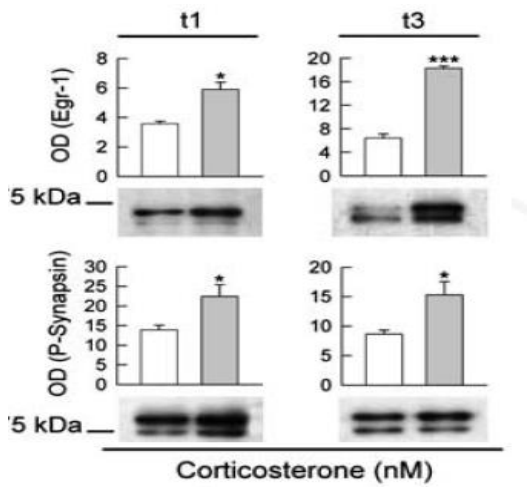
- Precellys®24 homogenizer Precellys® kit : 03961-1-003 (1.4mm ceramic beads).
- Samples: mice hippocampi and PC12 cells.
- Buffer: RIPA buffer containing protease and phosphatase inhibitors (Sigma).

/ PROTOCOL

Precellys®24: 5000 rpm, 2x30 sec, 10 sec break.
Centrifugation: 10000 rpm, 10 min, 4°C.
SDS-PAGE, immunoblotting with relevant antibodies.

/ RESULTS

The figure below shows an example of total protein extraction from PC12 cells using Precellys®24. Total extracts were analyzed by western blot. The corresponding X-ray films were quantified by densitometry. Those results allowed to better understand the action of corticosterone in the molecular cascade mediating the enhancement of stress-related memories.



Corticosterone-induced phosphorylation of synapsin-I depends on Egr-1 activation. Total extracts from PC12 cells were analyzed by western blot 1 and 3 h after treatment with 10nM corticosterone (gray bars). The corresponding X-ray films were quantified by densitometry (optical density, OD, mean± s.e.m., n = 3). *P < 0.05; ***P < 0.001, in comparison with basal levels.

/ CUSTOMER

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/ CONCLUSION

Precellys® is well adapted for extraction of total proteins from brain samples and cell lines. Sample homogenization is quick and efficient in term of quantity and quality of extracted proteins (e.g. phosphoproteins). With our protocol, we are able to treat 24 samples in less than 2 minutes by avoiding samples cross contamination.

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