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MODULATION OF THE L-TYPE CALCIUM CHANNEL CAV1.2 BY NOCICEPTIN AND ITS RECEPTOR NOP1

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INTRODUCTION

The endogenous neuropeptide nociceptin (N/OFQ) and its receptor, the nociceptin receptor (NOP1), are highly expressed in the hippocampus, where they regulate learning and memory by inhibiting synaptic transmission and plasticity. The L-type calcium channel (LTCC) Cav1.2 is also expressed in the hippocampus and is one of the major sources of Ca^{2+} in the postsynaptic compartment of neurons. Activation of Cav1.2 regulates memory and learning-associated biochemical changes through activation of cAMP response element-binding protein (CREB) and NMDA receptor-independent long-term potentiation (LTP) [1]. It has previously been shown that activation of NOP1 receptor by nociceptin downregulates LTCCs as well as other voltage gated calcium channels in hippocampal neurons [2], but little is known regarding the molecular mechanisms of this modulation.

METHODS

Co-immunoprecipitations were performed with transfected tsA-201cells and mouse hippocampal lysates. Cultured rat hippocampal neurons 10 days in-vitro were used to perform calcium imaging experiments using the Fluo-4AM dye and for confocal imaging of immunocytochemically stained cells for p-CREB and MAP2.

RESULTS

We show that Cav1.2 and NOP1 receptor form a complex and co-immunoprecipitate from transfected tsA201 cells and from hippocampal brain tissue. Calcium imaging of cultured rat hippocampal neurons treated with nociceptin (1uM) and depolarized with 40mM KCl revealed a significant decrease in calcium influx. Treatment with the LTCC blocker, Nifedipine showed that calcium influx is partially mediated by Cav1.2. Subsequent imaging of cultured rat hippocampal neurons treated with nociceptin (100nM) and depolarized with 40mM KCl revealed a significant decrease in CREB phosphorylation in the nucleus, an important step for neuronal plasticity. Treatments with Nifedipine and NMDA receptor antagonists (MK-801 and APV) indicate that the activation of nuclear p-CREB results in part from the activation of Cav1.2, and is NMDA receptor-independent.

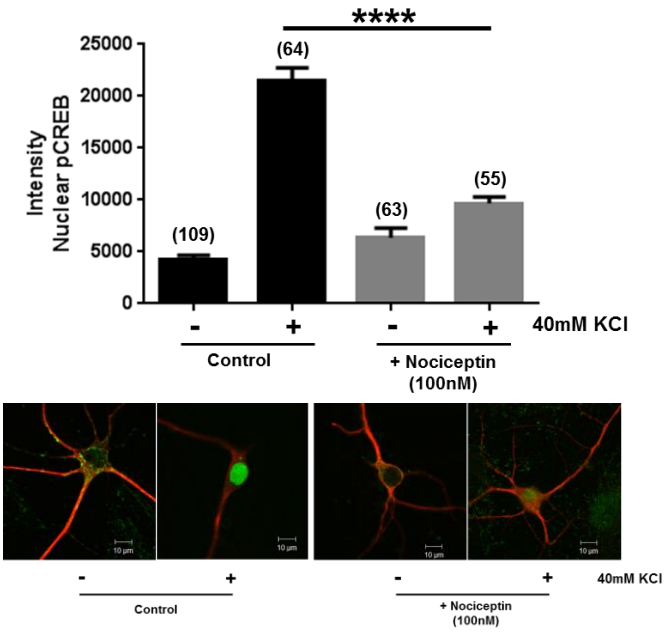

Figure 1. Nuclear p-CREB intensity as a function of drug and stimulatory treatment in cultured rat hippocampal neurons. Values represent the mean ± SEM. Sample size is indicated above each respective bar in parentheses (1-way ANOVA with Tukey post-hoc comparison, ****p<0.0001). Representative images of each treatment are listed below.

DISCUSSION AND CONCLUSIONS

Nociceptin and its receptor, NOP1, modulate the LTCC Cav1.2 and its downstream effects in the excitation-transcription pathway. Our data provide novel insights into the mechanisms by which the endogenous neuropeptide nociceptin affects hippocampal neuron function. Ultimately, this could identify new strategies for the alleviation of memory disorders. Further studies are being conducted to characterize the mode of Cav1.2 modulation and changes in its intrinsic properties.

REFERENCES

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2. Bongsebandhu-phubhakdi & Manabe. *J Neurosci.* **27**:4850-4858, 2007.