Premi de recerca per a estudiants
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Entrega de l'Abstract

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Títol de la recerca: **The role of glycinergic neurons in the process of somatosensory stimuli**
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THE ROLE OF GLYCINERGIC NEURONS IN THE PROCESS OF SOMATOSENSORY STIMULI

AIM

Test the role of glycinergic inhibitory neurons of the spinal cord in the process of somatosensory stimuli by checking the behavioural and histological changes in animals where these cells have been specifically killed.

MATERIALS AND METHODS

Viral mediated ablation of glycinergic neurons

Adult male BAC transgenic mice expressing eGFP, Cre recombinase and LacZ under the control of the GlyT2 promoter were injected with the viral vector pAAV.dflox.DTA.

The vector contained the Diphtheria Toxin A (DTA) reading frame inverted between two heterotypic loxP sites. Thus, viral transgene expression could only occur in Cre recombinase-expressing cells. After expressing DTA, the synthesis of new proteins is inhibited and therefore glycinergic neurons die.

Behavioural studies

Mice behaviour was studied the three days after the injection, assessing if they developed mechanical sensitivity or thermal hyperalgesia. The procedure consisted in measuring the pressure needed to induce a flexor response and the paw withdrawal latencies on exposure to a defined radiant heat stimulus.

Immunohistochemical studies

The fourth day after the intervention, animals were euthanized and we proceeded with the immunohistochemical study of their spinal cords.

Primary antibodies used were anti-Pax2, anti-GFAP and anti-IBA1. The analysis of sections stained with Pax2 and also of the eGFP fluorescence was used to quantify the loss of glycinergic neurons.

The inflammatory process caused by the intervention was quantified with the signal obtained with antibodies against GFAP and Iba1.

Results obtained with Cre positive mice were compared to ones of their wild type littermates.

RESULTS

We report the firsts results obtained after designing the procedure. Although we started with 11 animals, 3 of them had to be excluded of the study and therefore the following results are from 8 adult male BAC transgenic mice, 6 Cre positive and 2 wild type.

Viral mediated ablation of glycinergic neurons

The observation of eGFP expression in both animal groups confirmed that viral injections had efficiently and selectively killed glycinergic neurons.

Behavioural studies

Mice expressing Cre recombinase showed a reduction in paw withdrawal threshold after mechanical stimulation. Regarding the response to thermal stimuli, Cre+ animals had lower withdrawal latency than wild type, although the effect did not appear immediately.

Immunohistochemical studies

We observed an extensive loss of inhibitory neurons in Cre+ animals. When we quantified the inflammatory process, we detected more damage and inflammation in the injection side of Cre+ animals.

CONCLUSIONS

- Virus mediated ablation of glycinergic neurons works.
- After the loss of glycinergic neurons, the threshold for the paw withdrawal after mechanical or thermal stimuli shows a decreasing pattern.
- The inflammatory process after the injection is bigger in Cre+ animals.
- Further investigation needs to be made: the observed behaviour is caused by the loss of glycinergic neurons or by the inflammatory process?

***Personal contribution to the project**

My work consisted mainly in the immunohistochemistry procedures and the data analysis. Although I was not responsible for them, I also attended to several surgeries (virus injection or animal euthanasia and spinal cord harvest) and behaviour studies.