Opioid-mediated control of pain modulation from the medullary dorsal reticular nucleus: a gene therapy and pharmacological study in the monoarthritic rat

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Introduction

Chronic inflammation affects the activity of the supraspinal endogenous pain modulatory system, with enhancement of facilitatory actions[1,2] and alterations of the opioidergic system, such as decrease in the expression of opioid receptors[3]. An important component of the pain modulatory system is the dorsal reticular nucleus (DRt), an area involved in facilitation of nociceptive transmission from the spinal cord[4].

Gene therapy using Herpes Simplex Virus type 1 (HSV-1) has been used in acute[5] and chronic pain[6] control based in peripheral HSV-1 administration. Gene therapy-based treatments directed to the supraspinal endogenous pain modulatory system was seldom evaluated and only in acute pain conditions[7]. With the aim of inhibiting the DRt using gene therapy, we recently performed a study of the dynamics of migration of a HSV-1 vector containing the *lacZ* transgene, under the control of the human cytomegalovirus promoter (hCmv)[8]. Upon DRt injection, β-galactosidase (β-gal) stained neurons were detectable until 14 days post-injection, with maximal numbers of β-gal neurons obtained at 2 and 4 days in the DRt and transduction of several DRt afferents at 7 and 10 days post-injection.

In the present study, we evaluated the behavioural effects of local administration of a HSV-1 vector containing the human pre-proenkephalin gene (DPE), under the control of the hCmv promoter, using monoarthritic animals. We compared the data with pharmacological opioidergic manipulation of the DRt, by local microinjection of opioid receptor agonists [D-Ala2, Glu4]deltorphin (DELT) and [D-Ala2, NMePhe4Gly-ol5]enkephalin (DAMGO).

Methods

Male Wistar rats received an intraarticular injection of 50 µl of saline (saline group) or complete Freund´s adjuvant (CFA, monoarthritic group).

Gene therapy study:

→ Fourteen days latter, both animal groups were injected into the DRt with 2µl of HSV-1 containing the pre-proenkephalin (DPE virus) or the lacZ (DPZ virus) transgenes. Both vectors were used at the 2×10^8 PFUs concentration [n(Saline+DPZ)=4; n(saline+DPE=7); n(CFA+DPZ)=11; n(CFA+DPE)=12].

→ Thermal nociceptive thresholds were evaluated by determining paw withdraw latencies (PWL) at 2, 4, 7, 10 and 14 days after viral injection. Mechanical nociceptive threshold was accessed with von Frey filaments. A one-way ANOVA followed by the Student Neuman-Keuls (SNK) *post-hoc* test was used at each time point for statistical analysis.

→ Detection of the viral expression of human PPE was performed using additional animals which were injected with 5 µl of colchicine into the third (10 µg/µl) and fourth (20 µg/µl) ventricles 24 hours prior to sacrifice, which occurred 2, 4 or 7 days post-DPE injection.

→ Immunocytochemical detection was accomplish using a mouse anti-human PPE antibody (PE-21, B. Sproust, Dept of Anat & Physiol, University of Dundee, UK) and the ABC method.

Pharmacological study:

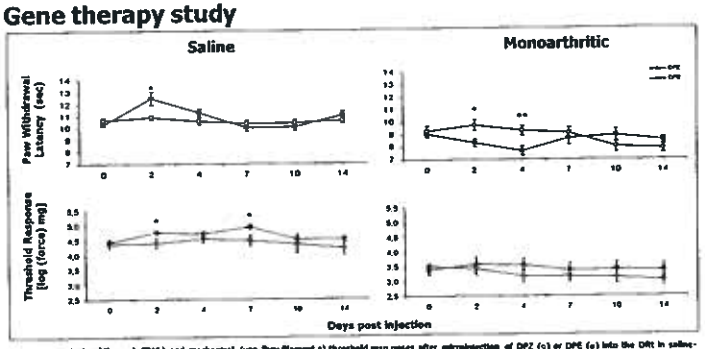
→ Seven days after intraarticular injections, animals were implanted with a guide cannula in the DRt.

→ Seven days latter, 0.6 µl of Saline, DELT (1.2, 12, 120 and 1200 ng) or DAMGO (0.1, 1, 25 and 50 ng) were injected into the DRt [n(saline+DELT)=8; n(CFA+DELT)=10; n(saline+DAMGO)=8; n(CFA+DAMGO)=10]

→ PWL were determined at 15, 30, 45 and 60 minutes after DRt injections. One-way ANOVA and SNK *post-hoc* test were used in statistical analysis.

Results

➢ Gene therapy study



Time-course study of thermal (PWL) and mechanical (von Frey filament s) threshold resp onses after microinjection of DPZ (○) or DPE (●) into the DRt in saline-injected or monoarthritic animals. Time 0 represent s the baseline thresholds determined before viral injection. The data of von Frey tests are presented in log(force x1 000)(-) - p <0.05, (**) - p<0.01.

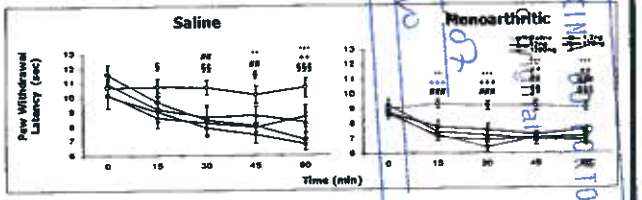
- **Thermal responses**: DPE injection significantly increased PWL at 2 days post-injection in saline-injected animals, whereas the opposite occurred in monoarthritic animals at 2 and 4 days.
- **Mechanical responses**: DPE significantly increased the responses to von Frey filaments at 2 and 7 days post-injection in saline-injected animals, but had no effect in monoarthritic animals.

Photomicrographs of brainstem sections showing neurons immunoreactive for human pre-pro-enkephalin 4 days after DPE injection at the nucleus of the solitary tract (A) and rostroventromedial medulla (B).

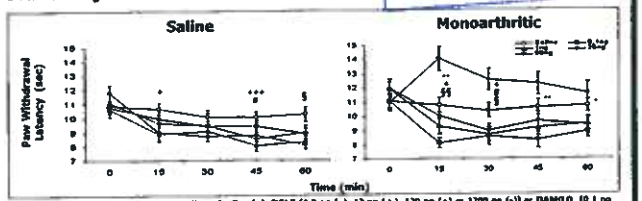
- Two days after DPE injection, high numbers of transduced neurons were detected at the caudal ventrolateral medulla, dorsal reticular nucleus, cuneate nucleus, vestibular nucleus, lateral paragigantocellular nucleus, parabrachial nucleus and medial cerebellar nucleus. At 4 days post-injection, the numbers of transduced neurons increased, decreasing at 7 days post-injection.
- In the hypothalamus a few transduced neurons were detected, with no relevant changes in numbers over time.

➢ Pharmacological study

➢ DELT injection



➢ DAMGO injection



Time-course study of PWL after microinjection of saline (○), DELT (1.2 ng (△), 12 ng (△), 120 ng (●) or 1200 ng (●)) or DAMGO (0.1 ng (○), 1 ng (△), 25 ng (●) or 50 ng (●)) into the DRt of saline-injected and of monoarthritic animals. Time 0 represent s the baseline value s determined before opioid microinjection. Symbols above the saline curves (○) represent statistical significance of each dose compared with saline at the corresponding time. (●) - 1.2 ng DELT and 0.1 ng DAMGO; (●) - 12 ng DELT and 1 ng DAMGO; (#) - 120 ng DELT and 25 ng DAMGO; (§) - 1200 ng DELT and 50 ng DAMGO. One, two or three symbols represent p< 0.05, p<0.01 and p<0.001, respectively.

- **DELT** decreased PWL in saline-injected and monoarthritic animals, an effect that was more pronounced in the latter experimental group.
- **DAMGO** decreased PWL in saline-injected animals. In monoarthritic animals, DAMGO also decreased PWL at lower doses (0.1, 1 and 25 ng) but induced analgesia at 50ng.

Conclusions

✓ The present result suggest that the effects of HSV-1 are due to transgene expression both at DRt neurons and in brain afferents.

✓ Differences between non-inflamed and inflamed animals indicate the existence of plastic changes induced by chronic pain, which appear to be different in distinct test modalities.

✓ Gene therapy may be an important strategy for manipulation of the supraspinal pain modulatory system by combining local correction of the effects of chronic pain in opioid receptor systems with directioning of viral expression to relevant brain areas.

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