Neurotrophins and their receptors, novel therapeutic targets for pelvic pain in endometriosis, are coordinately regulated by interleukin-1β

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Introduction

Pelvic pain manifested in women with endometriosis is attributed to afferent nociceptors and neuroinflammation in ectopic and eutopic endometrium.

Methods

We tested the hypothesis that nociceptive endometrial nerves were activated by IL-1β, a prominent cytokine in endometriosis, to influence expression of endometrial neurotrophins and their cognate receptors in vivo. Immunofluorescence histochemistry was used to confirm the presence of neurons in human endometrial tissue.

Fig.1

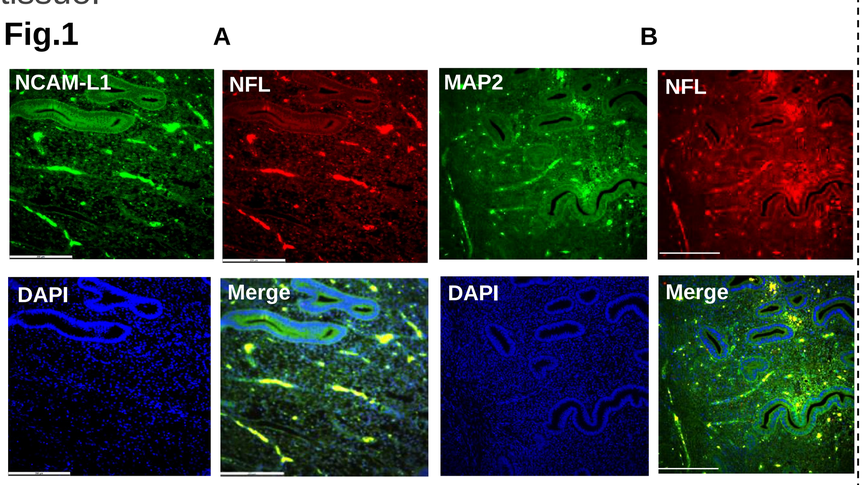

Fig.1 Immunofluorescence histochemistry (IFH) shows NCAM-L1, MAP2 (green) and NFL(red) positive fibers coursing through the stroma between epithelial glands of the endometrium functionalis layer.

Fig.2

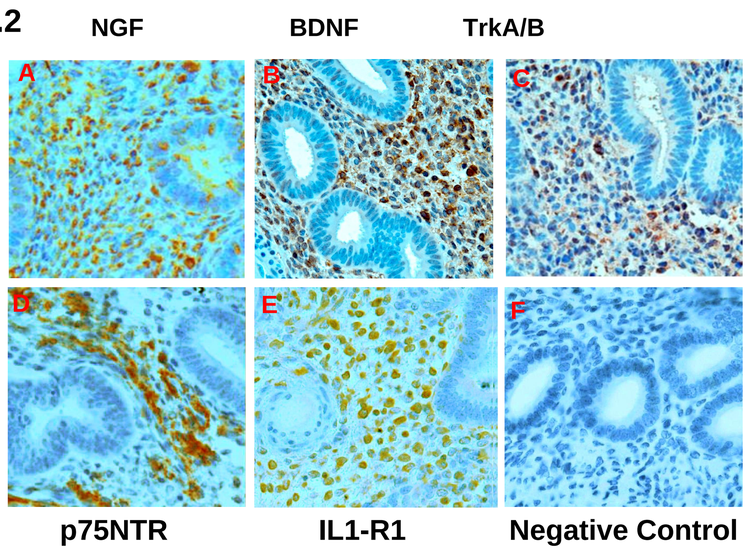

Fig.2 Immunohistochemistry of neurotrophins and their receptors expressed in human uterus: (A) NGF, (B) BDNF, (C) TrkA/B, (D) p75NTR-positive nerve fibers distribute mostly in the stroma and extend between glands within superficial endometrium. (E) Interleukin-1β receptor (IL1-R1) and (F) a negative control are included.

Fig.3

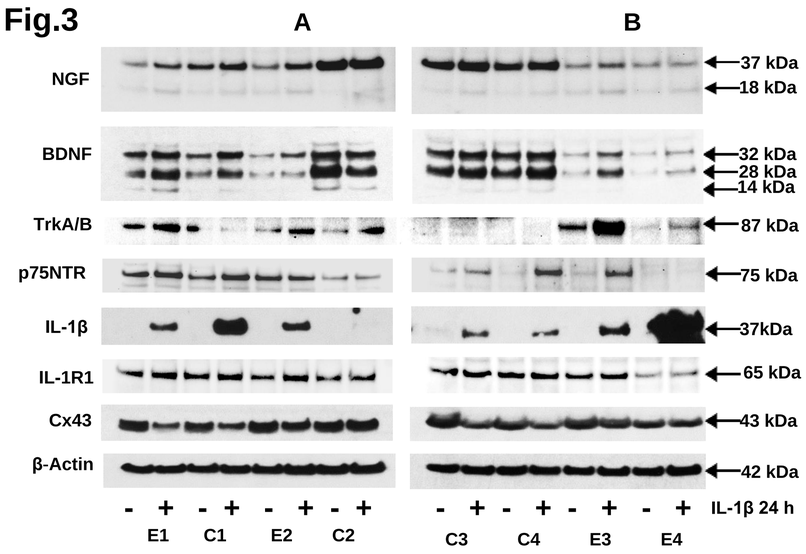

Fig.3A and B. ESC derived from 8 independent participants with (E1-E4) or without (C1-C4) endometriosis were analyzed. NGF, BDNF, TrkA/B, p75 NTR, IL-1β and IL-1R1 (respectively, were all upregulated by IL-1β after 24 hours treatment.

Fig.3C

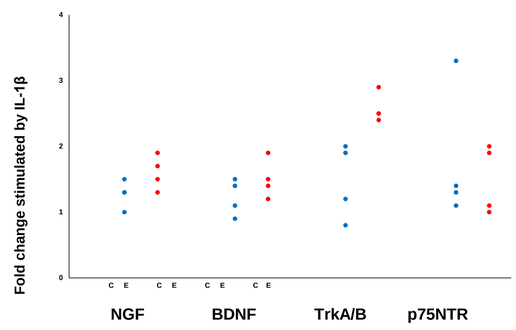

Fig.3C Scattergram of ESC neural protein responses to IL-1β stimulation. Results from ESC derived from controls (C, blue symbols, n=4) and endometriosis cases (E, red symbols, n=4) are shown.

Fig.4

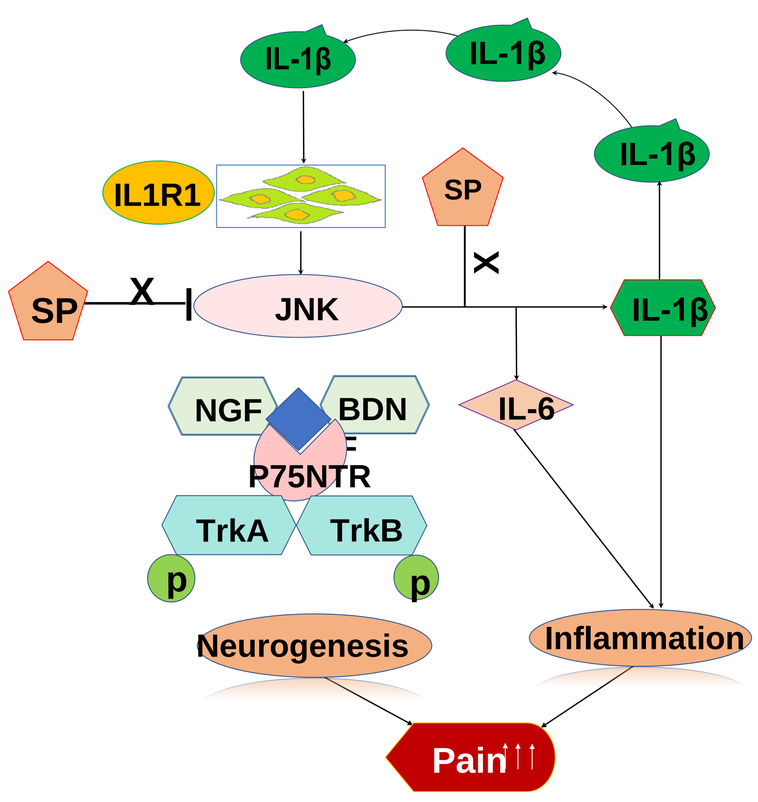

Fig 4. Graphical abstract of neuroinflammation in endometriosis

Fig.5

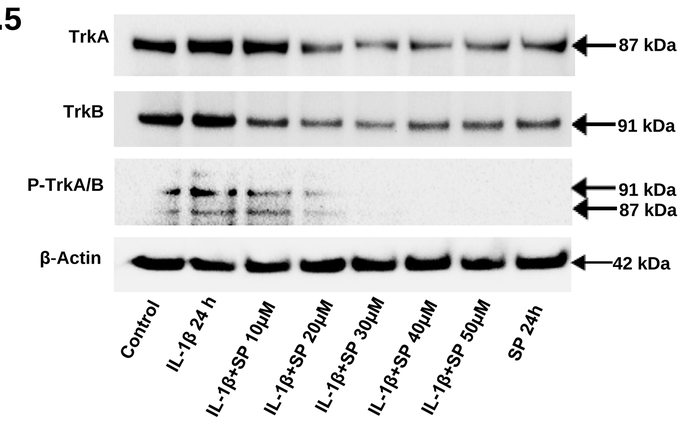

Fig. 5 Dose-response experiments with the JNK inhibitor SP600125 ("SP") were carried out in IL-1β-treated and control ESC. TrkA, -B and phosphorylated TrkA/B were all modestly stimulated by IL-1β.

Fig.6

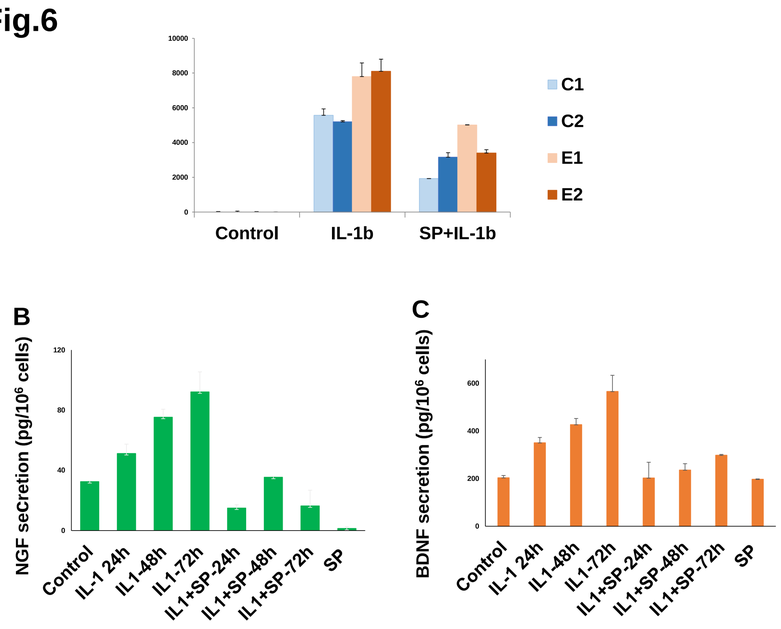

Fig. 6 (A) IL-6, (B) NGF, and (C) BDNF were measured by ELISA in ESC supernatants before (control) and after (IL-1) cytokine stimulation for 24-72 h.

Results

Expression of NGF and BDNF and their receptors in endometrial tissue and cells was validated by immunohistochemistry and Western blotting. Isolated endometrial stromal cells (ESC) were subjected to dose-response and time-course experiments in the absence or presence of IL-1β as an inflammatory stimulus. Specific kinase inhibitors were used to characterize the predominant pathways. In vitro biomarkers were quantified. Several neural biomarkers were co-localized in endometrial nerve fibers. NGF, BDNF and their receptors TrkA, TrkB and p75 NTR were all expressed in primary ESC. IL-1β stimulated higher TrkA/B expression in ESC derived from endometriosis cases (2.8 ± 0.2-fold) than cells from control subjects (1.5 ± 0.3-fold, t-test, P<0.01).

Fig.7A

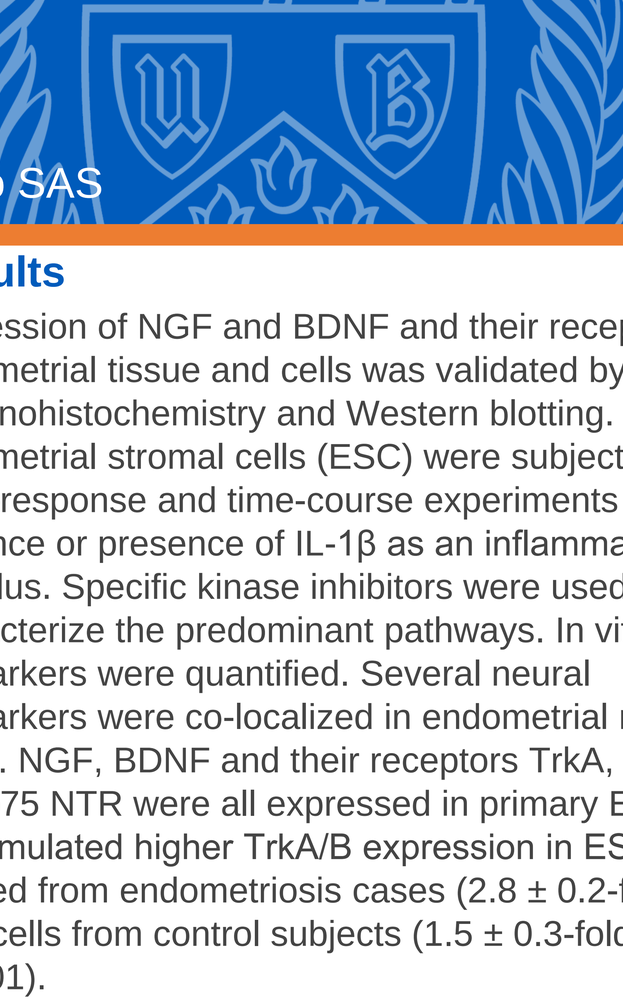

Fig. 7A Recombinant IL-1β treatment of ESC for 24-72 h increased, whereas coincubation with 30 μM SP at each time point reduced, neurotrophin and inflammatory proteins.

Fig.7B

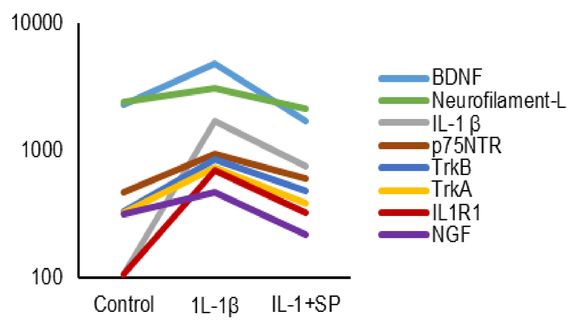

Fig. 7B Laser densitometry quantification of each of the 10 major protein bands in Fig. 7A, with the exception of β-actin revealed significant differences among the three treatment groups (P<0.01).

Conclusions

IL-1β stimulated NGF and BDNF, and also their cognate receptors, TrkA/B and p75NTR in ESC derived from endometriosis cases and controls. TrkA/B levels were greater in endometriosis cells than those from control subjects. The effects were mediated via an intracellular c-Jun N-terminal Kinase (JNK) cascade. Our results support the hypothesis that neurotrophins and their receptors orchestrate innervation of the endometrium, which is augmented by IL-1β. We postulate that JNK inhibitors, like SP600125, could be developed as therapeutic agents to reduce neuroinflammation in women with endometriosis-associated pain.