ERB-041, AN ESTROGEN RECEPTOR β AGONIST, AS AN ANALGESIC AGENT: CONTRIBUTION OF CENTRAL AND PERIPHERAL ERβ RECEPTORS

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Chronic inflammation is characterised by the development of hyperalgesia and allodynia to thermal and mechanical stimuli. Estrogen possesses anti-inflammatory properties possibly through modulation of the NF-κB pathway (Liu et al., 2005; Ghisletti et al, 2005) and therefore may have a role in modulating inflammatory hypersensitivity to pain. The aim of this study was to investigate the peripheral and central effects of the peripherally restricted ERβ agonist ERB-041 (Harris et al., 2004) in a model of established inflammatory hypersensitivity. The non-specific ER antagonist ICI-182780 was used to demonstrate that efficacy is via an ER dependant mechanism (Harris et al. 2003). In addition the effect of ERB-041 on cytokine release from the inflamed paw and primary spinal glia was determined in vitro. Male Random Hooded rats (180-240g) were used. 100μl of 1mg/ml Freund’s Complete adjuvant (FCA) was injected intraplantar into the left hind paw, whilst control animals received saline. 23 hrs later animals received either vehicle, ERB-041 20mg/kg p.o. once daily alone for 5 days, ICI-182780 25mg/kg s.c twice daily alone (8am, 8pm) or ICI-182780 25mg/kg s.c. b.i.d 30mins prior to ERB-041 and at 8pm for 4 days. The effect of ERB-041 on the FCA induced hypersensitivity was determined 1hr post dose on days 1, 2, 3 4 and 5. To determine the central action of ERB-041, 10μg of ERB-041 or vehicle was injected into the spinal cord under anaesthetic with recovery, 23hrs after intraplantar injection of FCA. The effect on the FCA induced hypersensitivity was determined 0.5, 1 and 1.5hrs post dose. The hypersensitivity to pain was determined using the dual channel weight averager (Clayton et al., 1997). To access the effect of ERB-041 on FCA induced cytokine release, paw samples were dissected and homogenised in PBS containing complete protease inhibitor cocktail. Homogenates were centrifuged, supernatants harvested and cytokine content was analysed by Luminex. Terminal paw samples were collected at day 5 following FCA injection (n=5). Cytokine release was also measured from rat primary microglial cultures to investigate a potential site of action. Primary microglia were stimulated to release pro-inflammatory mediators in medium containing 100ng/ml LPS and 1ng/ml IFN. Prior to cell stimulation, cells were pre-incubated with ERB-041 for 30 minutes. Cytokine release was assayed by Luminex. Data are presented as mean±s.e.m. Statistical analysis was carried out to determine whether there was a significant difference (p<0.05) between the vehicle and drug treated group using Fisher LSD (eFCA) or 2 tailed unpaired T-test (cytokine measurements.). In the FCA model daily oral dosing of ERB-041 fully reversed the FCA induced hypersensitivity. The effect was maximal 1 hr post dose on day 1. (98.0±12%, p<0.05) which was maintained throughout dosing. The antagonist ICI-182780 significantly reduced the anti-hypersensitive effects of ERB-041 throughout the four days of dosing. (ERB-041 max effect =25±11.5%, p<0.05) Following cessation of dosing of the antagonist (day 4) the anti-hypersensitive effects of ERB-041 returned (42.2±8.7%, P<0.05.). ERB-041 also significantly reduced FCA induced release from the paw of IL-1α (46%, p<0.05), IL-6 (35%, p<0.01) and TNF (19%, p<0.001) compared with the vehicle control. Intrathecal administration of ERB-041 (10μg) produced a significant reversal of the FCA induced hypersensitivity 30min post dose (51%±12.6%, p<0.05). ERB-041 (0.1-3μM) produced a dose related reversal of the LPS stimulated cytokine release from primary glia (max effect=46% at 3μM p<0.05). In conclusion: these data suggest that ERβ agonists may have clinical utility in the treatment of chronic inflammatory pain through a predominantly peripheral mechanism, but also suggests that targeting CNS ERβ receptors on e.g microglia may also have therapeutic benefit.