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Research Report

Anti-inflammatory substances can influence some glial cell types but not others

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ABSTRACT

In rat microglial enriched cultures, expressing Toll-like receptor 4, we studied cytokine release after exposure with 1 ng/ml LPS for 0.5–24 h. Dexamethasone and corticosterone exposure served as controls. We focused on whether naloxone, ouabain, and bupivacaine, all agents with reported anti-inflammatory effects on astrocytes, could affect the release of TNF-α and IL-1β in microglia. Our results show that neither ultralow (10^{-12} M) nor high (10^{-6} M) concentrations of these agents had demonstrable effects on cytokine release in microglia. The results indicate that anti-inflammatory substances exert specific influences on different glial cell types. Astrocytes seem to be functional targets for anti-inflammatory substances while microglia respond directly to inflammatory stimuli and are thus more sensitive to anti-inflammatory substances like corticoids. The physiological relevance might be that astrocyte dysfunction influences neuronal signalling both due to direct disturbance of astrocyte functions and in the communication within the astrocyte networks. When the signalling between astrocytes is working, then microglia produce less pro-inflammatory cytokines.

1. Introduction

Microglia are considered as immunocompetent cells of the CNS and are activated during pathological events such as stroke, ischaemia, or brain trauma to cause a neuroinflammation (Kreutzberg, 1996). In the normal brain, microglia appear as highly branched or ramified cells and thought to be quiescent. Activation of microglia alters their ramified morphology to amoeboid and proliferative with migratory behaviour. Surface molecules are expressed, cytokines are released, and growth factor synthesis show an up-regulated immunophenotype (Kettenmann et al., 2011).

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Activated microglia are known to release pro-inflammatory cytokines, particularly tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and also nerve growth factors, nitric oxide and prostanoids (Chao et al., 1992). These substances are present during inflammatory reactions and they produce long-term pain or hyperalgesia. Antagonism or neutralization of these factors can reduce the pain (Marchand et al., 2005).

Several substances have been shown to exert anti-inflammatory properties especially in astrocytes at extremely low concentrations: naloxone, ouabain, and bupivacaine (Lundborg et al., 2010, 2011; Block et al., 2012). Extremely low doses, at picomolar concentrations, of opioid receptor antagonists, such as naloxone or naltrexone, have been shown to enhance the efficacy and specificity of morphine and related opioid analgesics in mice and postoperative patients (Crain and Shen, 2000). Extremely low doses of naltrexone inhibit the development of spinal morphine antinociceptive tolerance, and clinical studies demonstrated that this may be due to attenuated glial activation (Mattioli et al., 2010). Ouabain, a digitalis-derived glycoside is a well-recognized Na^+/K^+-ATPase inhibitor, especially pronounced at high concentrations. It also enhances LPS down-regulated iNOS activity in peritoneal macrophages (Sowa and Przewlocki, 1997). Ouabain, at extremely low concentrations, nanomolar and picomolar, stimulates Na^+/K^+-ATPase activity (Zhang et al., 2008), and activates complex signalling cascades in kidney cells (Holthouser et al., 2010), and in cardiac and smooth muscle cells (Manunta et al., 2010). Ouabain also decreases the release of IL-1β in astrocytes (Forshammar et al., 2011). Bupivacaine is known to block Na^+ channels at high concentrations and change the excitability of action potentials. Clinically, this drug has been used in the treatment of various inflammation-related conditions and diseases (Cassuto et al., 2006), and to treat long-term pain in both cancer and non-cancer patients (Deer et al., 2002). Later it has been observed that local anaesthetics possess anti-inflammatory properties through their effects on cells of the immune system. These agents also attenuate the release of the pro-inflammatory cytokines TNF-α and IL-1β from intestinal cells (Lahav et al., 2002; Bedirli et al., 2011).

The purpose with the present study was to investigate if a number of non-steroid anti-inflammatory substances, administered within a wide dose range (10^{-12} M to 10^{-6} M) influence microglial release of pro-inflammatory cytokines.

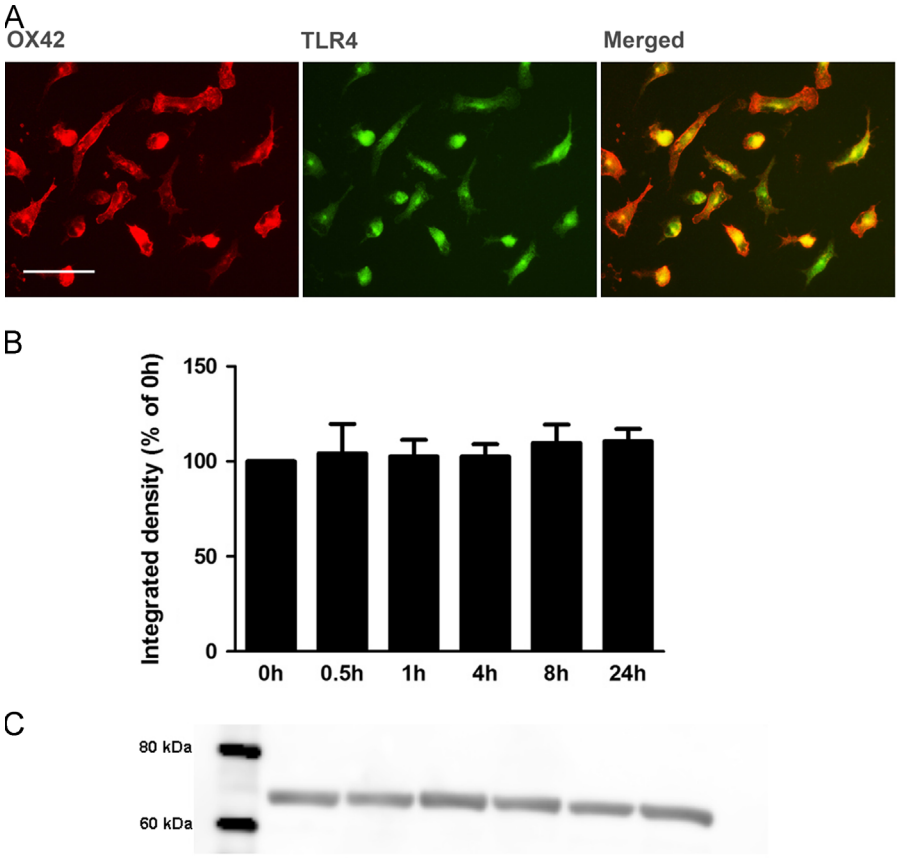

Fig. 1 – (A) Microglial cultures were stained with antibodies against OX42, or with antibodies against TLR4, revealing that the cells are microglia expressing TLR4 receptors. The two antibodies are then merged. (B) Microglia express TLR4 visualised with Western blot. Cultures were incubated with 1 ng/ml LPS for 0.5, 1, 4, 8, or 24 h. The TLR4 expression is shown as integrated density. (C) TLR4 is also shown as a band at ~70 kDa, reflecting the time exposure to LPS. Statistical analysis: The level of significance was analysed using one way ANOVA followed by Dunnett's multiple comparisons test. No significances were found.