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P2X7 is involved in the anti-inflammation effects of levobupivacaine

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ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form
30 June 2014
Accepted 10 July 2014
Available online 16 July 2014

Keywords:
Levobupivacaine
Microglia
Purinergic P2X7 receptor
Cell viability

ABSTRACT

Background: We sough to elucidate whether purinergic P2X7 receptor is actively involved in the effects of levobupivacaine on inhibiting microglia activation.

Materials and methods: Microglia were treated with lipopolysaccharide (LPS, 50 ng/mL), LPS plus levobupivacaine (50 μM), or LPS plus levobupivacaine plus the P2X7 receptor agonist Bz-ATP (100 μM) and denoted as the LPS, LPS + Levo, and LPS + Levo + Bz-ATP group, respectively. Microglia activation was measured by assaying inflammatory molecules expression. Microglia activation was also measured by assaying neuronal cell viability using coculture of microglia and neurons, as activated microglia may cause neuron injury. We also measured the levels of P2X7 receptor activation in microglia using ethidium uptake assay.

Results: Our data confirmed the effects of levobupivacaine on inhibiting inflammatory molecules upregulation in activated microglia, as the concentrations of interleukin (IL)-1β, tumor necrosis factor α, IL-6, and macrophage inflammatory protein 2, of the LPS + Levo group were significantly lower than those of the LPS group (all $P < 0.05$). Moreover, Bz-ATP significantly abrogated the inhibitory effects of levobupivacaine, as concentrations of IL-1β, tumor necrosis factor α, IL-6, and macrophage inflammatory protein 2 of the LPS + Levo + Bz-ATP group were significantly higher than those of the LPS + Levo group (all $P < 0.05$). In contrast, neuronal cell viability of the LPS + Levo group was significantly higher than those of the LPS and LPS + Levo + Bz-ATP groups ($P = 0.012$ and 0.002). Moreover, levels of P2X7 receptor activation of the LPS and LPS + Levo + Bz-ATP groups were significantly higher than that of the LPS + Levo group ($P = 0.003$ and 0.006).

Conclusions: P2X7 receptor is involved in the effects of levobupivacaine on inhibiting microglial activation.

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http://dx.doi.org/10.1016/j.jss.2014.07.020

1. Introduction

Levobupivacaine is an amino amide group local anesthetic agent that possesses similar potency to its racemic parent bupivacaine [1]. Previous data indicated less cardiac and central nervous system (CNS) toxicity of levobupivacaine compared with bupivacaine [1]. Clinically, levobupivacaine has emerged as a safer alternative for bupivacaine [1]. Previous data also highlighted the potent anti-inflammation effects of levobupivacaine. For example, levobupivacaine could inhibit vascular flare response induced by bradykinin and substance P in human skin [2]. Rectal administration of levobupivacaine could mitigate colon lesion in rats with colitis [3]. Our recent data further confirmed that levobupivacaine could inhibit microglia activation induced by endotoxin [4]. However, the mechanism(s) underlying the effects of levobupivacaine on inhibiting microglia activation remains to be elucidated.

Microglia are the resident immune cells of the CNS [5]. Activation of microglia constitutes a crucial CNS defense mechanism against invading pathogens [5]. However, microglia can in turn cause injury to neurons when activated in CNS infectious diseases [6–8]. The mechanisms involve the inflammation process triggered by activated microglia [6–8]. Previous data demonstrated that modulating the inflammation process triggered by activated microglia could provide beneficial effects against CNS infectious diseases [8,9]. Purinergic P2X7 receptor, a family member of the ionotropic ATP-gated purinergic receptors, plays a crucial role in regulating proliferation and activation of microglia [10,11]. Previous data also highlighted the active involvement of P2X7 receptor in mediating endotoxin-induced microglia activation [12,13].

Judging from these data, we speculated that levobupivacaine may very likely act through inhibiting P2X7 receptor to exhibit its effects on inhibiting endotoxin-induced microglia activation. To elucidate further, we thus conducted this cellular study. Our hypothesis was that augmenting P2X7 receptor activation could abrogate the effects of levobupivacaine on inhibiting endotoxin-induced microglia activation.

2. Materials and methods

2.1. Cell culture and cell activation protocols

BV-2, an immortalized murine microglial cell line, can readily produce inflammatory molecules with endotoxin exposure [4]. This study thus used BV-2 cells to facilitate investigation. Using Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Life Technologies), BV-2 cells were grown and maintained in a humidified incubator (37°C) supplied with a gas mixture of 5% CO_2/95% air. Then, confluent microglia (BV-2) were stimulated with gram (negative) endotoxin (lipopolysaccharide, LPS, 50 ng/mL, *Escherichia coli* Serotype 0127:B8; Sigma–Aldrich, St. Louis, MO) to induce microglia activation. The dosage of LPS was determined according to our previous data that LPS at the dosage of 50 ng/mL could readily induce microglia activation and upregulate inflammatory molecules expression in BV-2 cells [4].

2.2. Experimental protocols

To elucidate the possible role of P2X7 receptor in mediating the anti-inflammatory effects of levobupivacaine, microglia were randomly allocated to receive LPS, LPS plus levobupivacaine (50 μM; Abbott Laboratories Ltd, Abbott Park, IL), or LPS plus levobupivacaine plus the potent P2X7 receptor agonist Bz-ATP (100 μM; Sigma–Aldrich) and designated as the LPS, LPS + Levo, or LPS + Levo + Bz-ATP group, respectively. Levobupivacaine and/or Bz-ATP were added immediately after LPS. The dosage of levobupivacaine was determined according to our previous data that levobupivacaine at the dosage of 50 μM could consistently inhibited upregulation of inflammatory molecules in endotoxin-activated microglia and, most of all, posted no significant effects on microglia cell viability in endotoxin-activated microglia [4]. The dosage of Bz-ATP was determined according to previous data that Bz-ATP at the dosage of 100 μM could significantly activate P2X7 receptor [14].

To serve as controls for the additives, another set of microglia were randomly allocated to receive phosphate-buffered saline (PBS; Life Technologies), PBS plus levobupivacaine (50 μM), or PBS plus levobupivacaine plus Bz-ATP (100 μM) and designated as the PBS, Levo, or Levo + Bz-ATP group, respectively. In addition, another set of microglia were randomly allocated to receive PBS, PBS plus Bz-ATP (100 μM), LPS (50 ng/mL), or LPS plus Bz-ATP (100 μM) (designated as the PBS, Bz-ATP, LPS, or LPS + Bz-ATP group, respectively) to help confirm the effects of P2X7 receptor activation on modulating endotoxin-induced microglia activation.

2.3. Inflammatory molecules measurements using enzyme-linked immunosorbent assay

We assayed the concentrations of inflammatory molecules to determine the levels of microglia activation, as microglia will readily produce inflammatory molecules, including interleukin (IL)-1β, tumor necrosis factor α (TNF-α), IL-6, and macrophage inflammatory protein 2 (MIP-2), on exposure to endotoxin [4]. For assaying inflammatory molecules, BV-2 cells were plated on six-well dishes ($1–2 \times 10^6$ cells per well; Corning, Acton, MA). After reaction for 24 h, culture media from each group were harvested and then analyzed for the concentrations of IL-1β, TNF-α, IL-6, and MIP-2 using enzyme-linked immunosorbent assay (enzyme-linked immunosorbent assay kits of IL-1β, TNF-α, IL-6, and MIP-2; R&D Systems, Minneapolis, MN).

2.4. Analysis of nuclear factor κB activation using immunoblotting assay

We also assayed nuclear factor κB (NF-κB) expression to determine the levels of microglia activation, as expression of inflammatory molecules is tightly regulated by the upstream transcription factor NF-κB [15]. To assay NF-κB activation, BV-