Biochemical and Biophysical Research Communications 504 (2018) 513–518

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ropivacaine impairs mitochondrial biogenesis by reducing PGC-1α

Zejun Niu [a], Jiaming Tang [a], Yueyi Ren [b], Wei Feng [a, *]

[a] Department of Anesthesiology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China
[b] Heart Center, Women and Children's Hospital, Qingdao University, Qingdao, Shandong, 266034, China

ARTICLE INFO

Article history:
Received 26 August 2018
Accepted 29 August 2018
Available online 7 September 2018

Keywords:
Ropivacaine
PGC-1α
Mitochondrial biogenesis
Neurotoxicity

ABSTRACT

Ropivacaine is one of the commonly used local anesthetics in medical and dental care. However, preclinical and observational studies indicate that ropivacaine could have substantial side effects including neurotoxicity, which has raised concern regarding the safety of this drug. In the present study, we investigated the effects of clinically relevant doses of ropivacaine on mitochondrial biogenesis and function in neuronal cells. Our data indicate that exposure to ropivacaine leads to reduced expression of the major mitochondrial regulator PGC-1α and its downstream transcription factors NRF1 and TFAM. Ropivacaine treatment induces impairment of mitochondrial biogenesis by reducing mitochondrial mass, the ratio of mtDNA to nDNA (mtDNA/nDNA), cytochrome C oxidase activity, and COX-1 expression. Additionally, treatment with ropivacaine causes "loss of mitochondrial function" by impairing the mitochondrial respiratory rate and ATP production. Mechanistically, the reduction of PGC-1α caused by ropivacaine exposure requires inactivation of CREB, while re-introduction of PGC-1α completely rescues ropivacaine-induced mitochondrial abnormalities. In summary, our results provide supporting evidence that mitochondrial impairment is a key event in ropivacaine-mediated neurotoxicity, and the reduction of PGC-1α and its downstream signals are likely the molecular mechanism behind its cellular toxicity.

1. Introduction

In medical and dental care, local anesthetics (LA) are often used to prevent or relieve pain in specific regions of the body. Local anesthetics act by reversibly blocking neuronal voltage-gated sodium channels and interrupting nerve conduction without the loss of consciousness [1]. Based on the chemical composition, clinical LAs can be categorized into two classes: amides and esters [2]. Ester LAs are quickly metabolized by the blood and tissue esterases and have very short half-lives, lasting for only a few minutes. Amide LAs are absorbed into the blood and distributed via circulation. They are hydrolyzed by liver microsomal enzymes. The half-lives of ester LAs are relatively long (2–3 h) and toxicity is more likely to occur in patients with impaired liver function [3].

Despite their clinical benefits, local anesthetics have been reported to exhibit time- and dose-dependent toxicity to a variety of tissues, including nerves. There are preclinical experiments and clinical evidence showing that local anesthetics have nontoxicity issues [4,5]. A previous study has shown that commonly used LAs including bupivacaine and ropivacaine could induce apoptosis of neuronal cells at the clinically used concentrations [6]. Both bupivacaine and ropivacaine are long-acting amide LAs. Bupivacaine, an anesthetic that has been widely used for more than half a century, has been shown to cause neurological damage in preclinical rodent experiments [7,8]. Ropivacaine was developed after bupivacaine and noted to be associated with cardiac arrest, particularly in pregnant women [9]. Ropivacaine was found to have less cardiotoxicity than bupivacaine in animal models [10].

Different mechanisms of action of ropivacaine have been proposed. For example, stress-responsive MAPK kinase activation is reported in both bupivacaine- and ropivacaine-induced neurotoxicity [11]. Another report showed that bupivacaine and ropivacaine could suppress nerve growth factor, a neuropeptide primarily involved in the regulation of the growth, maintenance, proliferation, and survival of certain types of neurons [12]. However, the mechanism of these LAs responsible for neurotoxicity remains elusive. In the current study, we investigated the effect of ropivacaine on cellular mitochondrial function in cultured neuronal cell lines.

* Corresponding author. Department of Anesthesiology, The Affiliated Hospital of Qingdao University, No 16 Jiangsu Road, Qingdao, Shandong, 266003, China.
E-mail address: wei02feng08@126.com (W. Feng).

https://doi.org/10.1016/j.bbrc.2018.08.186
0006-291X/

2. Methods and materials

2.1. Cell culture and ropivacaine treatment

We purchased BE (2)-M17 neuronal cell line from ATCC (CRL-2267™), hereinafter referred to as M17. The cells were cultured in complete media with a 1:1 mixture of Eagle's Minimum Essential Medium and F12 Medium supplemented with 10% fetal bovine serum. To test the effect of ropivacaine on neuronal cells, ropivacaine solution was mixed with cell culture media to make 0.25%, 0.5% and 1% concentrations. The cells were treated with ropivacaine for 1 h in the CREB inactivation experiment. For other experiments, cells were treated for 24 h or 48 h. For the cAMP experiment, cells were treated with 10 μM cAMP for 24 h. We purchased PGC-1α adenovirus from Addgene, USA. To infect M19 cells, adenovirus was added to 50% confluent cells for 24 h at 10 MOI. The infection efficiency was assessed by immunoblotting with PGC-1α antibody.

2.2. Real time PCR analysis

We extracted total RNA from cultured cells using a high pure RNA kit from Roche (#12033674001). RNA concentrations were quantified using a Nanodrop spectophotometer. A total of 1 μg of intracellular RNA was used to synthesize the cDNA with iScript Supermix from Invitrogen. The SYBR-based real-time PCR experiment was performed to detect the mRNA transcript of PGC-1α, NRF1, and TFAM on an ABI 7500 platform.

2.3. Western blot analysis

M17 cells under different conditions were lysed by RIPA buffer with protease inhibitor. A total of 20 μg of cell lysates was loaded onto 4–20% precasted PAGE gel to separate the proteins by size. The separated protein mixes were transferred to PVDF membranes to detect the corresponding protein levels using specific antibodies. The following antibodies were used: PGC-1α, NRF1, TFAM, CREB, p-CREB, COX-, and β-actin.

2.4. Mitochondrial staining and mass

We purchased MitoTracker Red dye from ThemoFisher (M7512). Briefly, cells on cover slides were fixed with 4% PFA and permeabilized with 0.3% PBST, and MitoTracker dye was added to the fixed cells to stain mitochondria for 15 min. The stained mitochondria in 10 randomly selected fields were visualized and calculated.

2.5. Mitochondrial and nuclear DNA ratio (mtDNA/nDNA)

We purchased a Human Mitochondrial to Nuclear DNA Ratio Kit from EMD (cat #72620). Real-time PCR-based assays were performed to compare the mtDNA copy number to nuclear DNA (nDNA). The ratios were calculated based on the average number of two sets of mtDNA and nDNA genes.

2.6. Measurement of mitochondrial respiration

After the indicated treatment, O2 consumption in M17 cells was evaluated using a respirometer with a Peltier thermostat and electromagnetic stirrer. Briefly, 1 ml culture medium containing 5×106 M17 cells was put in a glass chamber equilibrated in ambient room air with continuous stirring (750 rpm) for 10 min. The oxygen concentration was recorded at 2 s intervals.

2.7. Measurement of intracellular ATP

To measure the cellular metabolic energy source, we measured total ATP levels from cells under different conditions. The colorimetric assay-based kit used in this experiment was purchased from Life Technologies (USA). Cellular ATP concentrations were quantified according to the standard curve.

2.8. Statistical analysis

All experimental data are shown as means ± S.E.M from at least three separate experiments. The statistical significance of differences among different groups was assessed by one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ropivacaine treatment suppresses PGC-1α and its downstream transcription factors NRF1 and TFAM in neuronal cells

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is one of the master regulators of mitochondrial biogenesis. To examine the potential influence of ropivacaine on neuronal cell line M17 cells, we tested the effects of various clinically relevant concentrations of ropivacaine on PGC-1α. Our data show that the three tested concentrations of ropivacaine inhibited PGC-1α expression in a dose-dependent manner. At the mRNA level, 0.25%, 0.5%, and 1% ropivacaine exposure for 24 h led to reductions in PGC-1α of 28%, 46%, and 58%, respectively, as compared to non-treated cells (Fig. 1A). At the protein level, 0.25%, 0.5%, and 1% ropivacaine caused a similar reduction in PGC-1α of 25%, 39% and 51% as compared to non-treated cells. We then tested the two major transcription factors modulated by PGC-1α: NRF1 and TFAM. Indeed, the three different concentrations of ropivacaine had a dose-dependent influence on mRNA levels of NRF1 and TFAM (Fig. 1C), and ropivacaine exerted a very similar inhibition on the protein expressions of NRF1 and TFAM (Fig. 1D).

3.2. Ropivacaine treatment impairs mitochondrial biogenesis in neuronal cells

To assess the impact of ropivacaine treatment on mitochondria, we evaluated the patterns of mitochondrial biogenesis in M17 neuronal cells. Compared with non-treated cells, 0.5% ropivacaine exposure for 48 h caused a reduction in the total mass of mitochondria by an average of 34% as revealed by mitochondrial marker staining (Fig. 2A). Treatment with ropivacaine under the same conditions resulted in a reduction of approximately 31% in mtDNA/nDNA (Fig. 2B). Additionally, 0.5% ropivacaine treatment resulted in a 38% reduction in cytochrome C oxidase activity on average and a 49% reduction in COX-1 expression. These results suggest that ropivacaine has a negative impact on mitochondrial biogenesis.

3.3. Ropivacaine treatment causes "loss of mitochondrial function" in neuronal cells

We then tested the effect of ropivacaine exposure on mitochondrial function. When M17 cells were constantly exposed to media containing 0.5% ropivacaine for 48 h, time course monitory experiments showed that cellular oxygen consumption was significantly lower than in non-exposure cells (Fig. 3A). Upon ropivacaine exposure, the rate of cellular mitochondrial respiration was about 30% lower than under non-exposure conditions (Fig. 3B). Additionally, the level of mitochondrial ATP production in ropivacaine-treated cells was about 30% lower than in non-