Bupivacaine induces apoptosis via blocking T- type Ca2+ channels and resulting activation of mitochondrial apoptotic pathway in SH-SY5Y cells

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Back ground

Local anesthetics have been reported to be neurotoxic. Recently, we reported that bupivacaine induced neuronal apoptosis via blocking T-type Ca2+ channels, but the detailed mechanisms are still not clear. Since mitochondria play key roles in activating apoptosis in mammalian cells, we investigated the role of mitochondria in apoptosis induced by bupivacaine via blocking T-type Ca2+ channels.

Method

1. Cell culture

The SH-SY5Y human neuroblastoma cells were grown in MEM and Ham’s F12 nutrient mixture (MEM/F12 ; 1:1) containing 10% heat inactivated fetal bovine serum with 100 units/ml penicillin and 100 µg/ml streptomycin and maintained in a humidified 5% CO₂ incubator at 37°C. SH-SY5Y cells were treated with bupivacaine, NNC55- 0396 (selective T-type Ca2+ channel inhibitor), EGTA (a divalent cation chelater) and CaCl₂, then activation of caspase-3 was determined. In order to further illustrate whether mitochondria contribute to apoptosis, the JC-1 staining was used to evaluate the change of mitochondrial membrane potential (MMP) and production of intracellular reactive oxygen species (ROS) in bupivacaine-treated cells were also analyzed by flow cytometry.

3. Statistics

p<0.05 was considered to be statistically significant for analysis of variance or t-test.

Results

1. Bupivacaine activated caspase-3 in a time-dependent manner. EGTA further enhanced bupivacaine-induced caspase-3 activation at 16 h after treatment. However, CaCl₂ significantly decreased bupivacaine-induced caspase-3 activation(Fig. 1). NNC55-0396 not only activated caspase-3 but also enhanced bupivacaine-induced activation of caspase-3 at 8 h after treatment(Fig.2).

Fig 1.

Fig 2.

2. At 24 h after treatment, bupivacaine significantly reduced MMP. Bupivacaine-induced reduction in MMP was greatly increased by NNC55-0396 and EGTA, but significantly inhibited by CaCl₂. (Fig. 3,4)
3. Both bupivacaine and NNC55- 0396 significantly increased level of intracellular ROS. Furthermore, a combination of bupivacaine and NNC55-0396 greatly increased ROS production.(Fig. 5) Compared to EGTA, which enhanced the bupivacaine-induced ROS production at 16 h after treatment, CaCl₂ significantly inhibited such change. (Fig. 6)

Fig 3.

Fig 4.

Fig 5

Fig 6.

Discussion

- In SH-SY5Y cells, bupivacaine reduced MMP and increased intracellular ROS production, resulting in activation of caspase-3. Both NNC55-0396 and EGTA greatly enhanced bupivacaine-induced MMP depolarization, intracellular ROS accumulation and caspase-3 activation. However, CaCl₂ significantly suppressed these changes induced by bupivacaine.
- These results suggest that T-type Ca2+ channel is somehow involved in the process of bupivacaine-induced mitochondrial dysfunction. Inhibition of extracellular calcium influx may play an important role in this apoptotic pathway.

Conclusion

Mitochondrial dysfunction is critically involved in bupivacaine-induced apoptosis. Bupivacaine may induce cell death via blocking the T-type Ca2+ channel and resulting activation of mitochondrial apoptotic pathway.