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Neurotoxicity of local anesthetics: effects on growing neurites and growth cones

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Background

Local anesthetics have been suggested to have a potential for neurotoxicity, in both clinical reports and laboratory experiments. However, the precise morphological changes induced by the direct application of local anesthetics to neurons are not yet fully understood. Also, despite the fact that local anesthetics are sometimes applied to sites where peripheral nerves may be regenerating after injury, the effects of local anesthetics on growing or regenerating neurons have never been studied. Growing or regenerating neurons may be susceptible to the toxic effects of local anesthetics.

To examine the effects of local anesthetics on growing neurons, we adopted a growth-cone collapse assay, which is a quantitative measurement of neuronal morphological changes induced by externally applied substances [1,2]. The growth cone is the leading edge of an extending neurite, and growth cones have crucial roles in pathfinding and cytoskeletal organization during neuronal development [3]. By morphologically observing growth cones and growing neurites, the effects of externally applied substances on growing nervous tissues can be clearly identified.

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Results of laboratory experiments

Growth-cone collapse and neurite retraction by local anesthetics

In the first study, we examined the effect of the local anesthetics, tetracaine and bupivacaine, on three different types of growing neurons isolated from chick embryos [4]. Tetracaine induced growth-cone collapse and neurite destruction. The three types of neuronal tissues, retinal ganglion layer, dorsal root ganglion neurons and sympathetic neurons, showed significantly different dose-responses, both at 60 min and at 24 h after the application of tetracaine ($P < 0.01$). The growth-cone collapsing effect was partially reversible in dorsal root ganglion and retinal neurons; however, in the sympathetic ganglion culture, no reversibility was observed after exposure to 1 mM tetracaine for 10 or for 60 min. Bupivacaine, in contrast, had similar neurotoxicities in the three types of growing neurons.

In the second study, we compared the neurotoxic potentials of different local anesthetics in isolated dorsal root ganglion neurons [5]. We observed that all of the local anesthetics examined, lidocaine, bupivacaine, mepivacaine, and ropivacaine, produced growth-cone collapse and neurite degeneration. However, they showed significant differences in dose response. The IC (50) values were approximately 10 (–2.8) M for lido-

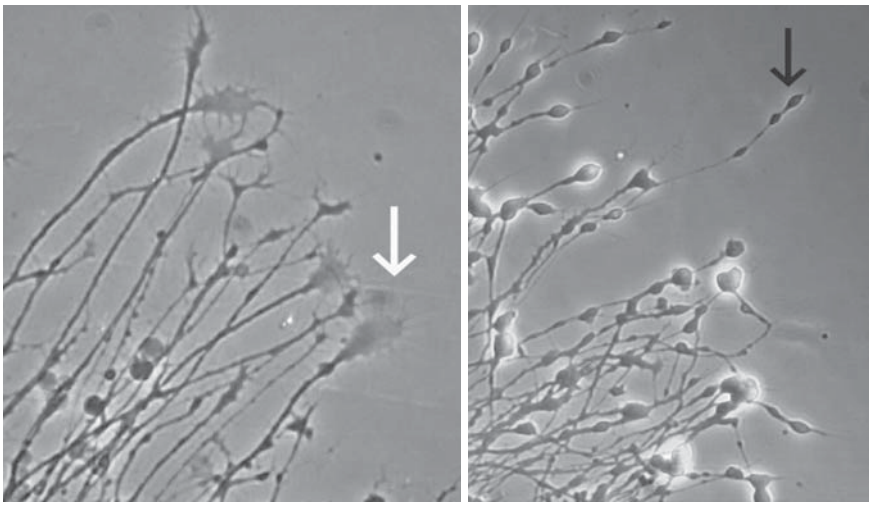

Fig. 1a,b. Growth-cone collapse induced by 0.1 mM tetracaine. **a** The *white arrow* indicates the intact growth cone prior to tetracaine exposure, and **b** the *black arrow* indicates a collapsed growth cone following the exposure

caine, 10 (–2.6) M for bupivacaine, 10 (–1.6) M for mepivacaine, and 10 (–2.5) M for ropivacaine at 15-min exposure. Some reversibility was observed after replacement of the media. At 20 h after washout, bupivacaine and ropivacaine induced minor growth-cone collapse in comparison to their control values, whereas lidocaine and mepivacaine induced significantly larger growth-cone collapse than the control values.

Supporting actions of neurotrophic factors

The above findings have generated interest in factors that could rescue neurons affected by the neurotoxicity of local anesthetics. Therefore, in the following study, we designed experiments to investigate the role of three neurotrophic factors (NTFs) in supporting developing neurons exposed to the deleterious effects of these drugs [6]. After 60 min of exposure to lidocaine, the culture media were replaced to wash out the lidocaine. When any of the three NTFs (brain-derived neurotrophic factor, glial-derived neurotrophic factor, or neurotrophin-3) was added to the replacement media at a minimum concentration of 10 ng·ml^{-1}, significantly high reversibility of the lidocaine-induced growth-cone collapse was observed, especially at 48 h after washout ($P < 0.05$). At that time point, there was no significant difference between the percentage growth-cone collapse in the cells that were exposed to lidocaine and supported by the NTFs after the washout, and the control cells (not exposed to lidocaine) ($P > 0.05$). Similarly, when any of the three NTFs were used after the washout of bupivacaine or mepivacaine, the growth-cone-collapsing activity was significantly attenuated, and growth-cone collapse values showed no statistically significant differences in comparison with the pre-exposure values obtained prior to the application of local anesthetics ($P > 0.05$) [7].

Intracellular mechanism of growth-cone collapse, and effects of lower anesthetic concentrations

To clarify the intracellular mechanism of growth-cone collapse and neurite retraction induced by local anesthetics, we examined whether tetracaine increased Ca^{2+} concentration during growth-cone collapse [8]. The intracellular Ca^{2+} concentration was measured by fura 2/acetoxymethyl (fura 2/AM) after exposure to tetracaine. Tetracaine (>1 mM) induced collapse and Ca^{2+} increase in the growth cones simultaneously ($P < 0.01$). The Ca^{2+} hot spot was expanded into the neurite from the periphery towards the cell body. When tetracaine was applied to growth cones in Ca^{2+}-free media, the increase was minor.

In our most recent study, the effects of prolonged exposure to a local anesthetic at a low concentration were studied [9]. Neurite growth 24 and 48 h after tetracaine application was delayed significantly when tetracaine was applied at a concentration higher than 5 µM. The filopodia of growth cones retracted, and their numbers were significantly decreased 24 and 48 h after the application of 10 and 20 µM tetracaine. The quantity of actin in the cell bodies increased, contrary to the effect on neurites and growth cones.

Conclusions and future perspectives

From our previous observations, the effects of local anesthetics on growing neurons were summarized as follows:

(1) Short-term exposure to tetracaine produced irreversible changes in growing neurons. Growth cones were quickly affected, and neurites subsequently degenerated. This phenomenon was related to intracellular Ca^{2+} increases expanding into the neurite

from the periphery towards the cell body. Sensitivity to the toxicity varied with neuronal subtypes.

(2) Toxicity to growing neurons may be common with all local anesthetics. However, the neurotoxic potencies were not identical for these drugs. The IC (50) values and reversibility of the morphological changes differed among the local anesthetics.

(3) Three neurotrophic factors (NTFs)—brain-derived neurotrophic factor, glial-derived neurotrophic factor, and neurotrophin-3—can, partly reverse the growth-cone collapse induced by local anesthetics. The effect was concentration- and time-dependent, and did not depend on the type of local anesthetic that induced the growth-cone collapse.

(4) Continuous exposure to tetracaine at low concentrations delayed neurite growth and disturbed the motility of growing neurites.

When applied to growing or regenerating neurons, local anesthetics delay or interrupt neurite extension. This effect can be deleterious for the normal establishment of nervous tissue. However, where abnormal sproutings provoke neurological disorder, this inhibitory action of local anesthetics might be beneficial for maintaining normal neuronal circuits [10]. This toxicological knowledge seems to be crucial for physicians when considering the future clinical applications of local anesthetics.

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