INTRODUCTION:
The extension of nerve fibers into the inner layers of degenerated discs is thought to cause discogenic pain.[1] The recent finding of the expression of tumor necrosis factor-α (TNF-α) in degenerated discs suggested its possible involvement in pain generation.[2] However, the direct effects of TNF-α on axonal growth of DRG neurons remains controversial.[3, 4]

Nociceptive dorsal root ganglion (DRG) neurons are classified into nerve growth factor (NGF)-sensitive and glial cell line-derived neurotrophic factor (GDNF)-sensitive neurons. Recent studies revealed that most disc-innervating neurons, which are suggested to be associated with discogenic pain, are NGF-sensitive.[5] To determine the axonal growth potential of both types of DRG neurons in response to axonal injury and TNF-α, an in vitro organ culture system was utilized for this study. The reliability of the organ culture system and the cell viability of the DRG neurons were also examined.

METHODS: Thirteen Sprague-Dawley rats (250-300g) were used. Bilateral lumbar DRGs from the L3 to L5 levels were removed and immediately fixed (non-cultured) or incubated free-floating in one ml of serum-free medium containing TNF-α. The extension of nerve fibers into the inner layers of degenerated discs is thought to cause discogenic pain.[1] The recent finding of the expression of tumor necrosis factor-α (TNF-α) in degenerated discs suggested its possible involvement in pain generation.[2] However, the direct effects of TNF-α on axonal growth of DRG neurons remains controversial.[3, 4]

To determine the axonal growth potential of both types of DRG neurons in response to axonal injury and TNF-α, an in vitro organ culture system was utilized for this study. The reliability of the organ culture system and the cell viability of the DRG neurons were also examined.

RESULTS: ATF3-IR or GAP-43-IR neurons in the non-cultured and cultured DRGs were rarely observed in the non-cultured DRGs (Figure 2). However, after 48 hours of cultivation, the number of GAP-43-IR neurons significantly increased in all of the cultured DRGs (Figure 2, p<0.05).

No significant difference was observed in the number of ATF3-IR and GAP-43-IR neurons among the control group and the TNF-α groups (all concentrations, Figures 1, 2).

Proportion of GAP-43-IR in CGRP- and IB4-positive neuronal profiles: The percentage of GAP-43-IR neurons in CGRP- and in IB4-positive neuronal profiles was significantly higher in cultured DRGs than in non-cultured DRGs (Figures 3, 4, p<0.05). The percentage of GAP-43-IR neurons in CGRP- and IB4-positive neuronal profiles in the TNF-α 1 ng/ml group was significantly higher than that in the control group of cultured DRGs (Figure 4, p<0.01). The percentage of GAP-43-IR neurons in CGRP-positive neuronal profiles was significantly higher than that in IB4-positive neuronal profiles at any concentrations of TNF-α (Figure 4, p<0.0001 - p<0.01).

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