INTRODUCTION

It has been reported that an extract of denervated muscle promotes in vitro neurite outgrowth of chick telecephalic and spinal neurons (Taguchi et al., 1987). To characterize these muscle-derived factors, a denervated crus muscle cDNA library was screened with an antibody to inhibit neurite outgrowth activity in vitro (Nishimune et al., 1998). Three neurite outgrowth factors were identified: neurocrescin (Nishimune et al., 1997), muscle-derived protein with molecular mass of 77kDa (MDP77; Uyeda et al., 2000, and MDP62 (Uyeda et al., 2000). Among them, the MDP77 gene was expressed during the period of neuromuscular junction development, and in the myotome at P5 when motoneurons send their axons from the ventral horn of the spinal cord. In this study, the long-term effects of rhMDP77 on nerve-tissue regeneration were examined.

MATERIALS AND METHODS

A 407 µg/ml rhMDP77 solution containing 50 mM Tris-HCl (pH 7.0), 150 mM NaCl, 1 mM EDTA, and 1 mM DTT was added to a 9.9 mg/ml type-I collagen solution derived from porcine dermis and DMEM buffer solution at 0°C. The concentration of collagen was adjusted to 0.2 mg/ml, and the concentration of rhMDP77 to 0, 5, 10, or 20 µg/ml. This mixed protein solution was injected into silicone tubes (15 mm in length, 2.5 mm in outer diameter and 1.5 mm in internal diameter), and gelated at 37°C for 30 min. The tubes were used for implantation and are referred to respectively as MDP-0, -5, -10, and -20 tubes. The right sciatic nerve of the SD rat was exposed and the silicon tubes were implanted.

The hind limbs of the rats were dipped in Indian ink. The rats were then allowed to walk on paper, leaving footprints on a white paper strip. The maximal toe-spread index (TSI) was calculated according to the following formula (Brown et al., 1989): TSI = (NTS - ETS) / NTS, where TSI = experimental spread in mm between first and fifth toes, ETS = terminal latency quotient between the implanted and non-treated side of the same rat with time. The hot-plate latency was recorded, and the average latency over three trials was recorded. These procedure was repeated every month for 6 months after the operation. Electrophysiological evaluations were carried out six months after implantation. Evoked muscle action potentials (EMAPs) were recorded on the tibialis anterior (TA) muscle in response to single stimulus pulses. The ratio of soleus muscle weight to body weight was calculated for each rat. The average number of S100-positive cells in the tube.