Membrane elasticity of mouse dorsal root ganglion neurons decreases with aging

Hidenori Horie[1], Shinichiro Ikuta[2] and Toshifumi Takenaka[1]

[1]*Department of Physiology, School of Medicine, Yokohama City University, 3-9 Fukuura, Kanazawaku, Yokohama (Japan)* and [2]*Department of Physics, Waseda University, Shinjuku-ku Tokyo (Japan)*

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Elastic area compressibility modulus (EACM) method was applied to measure the membrane elasticity of cultured dorsal root ganglion neurons from fetal and 3-month-old mice. The values of the EACM were 21.3 dyn/cm in 3-month-old neurons and 2.8 dyn/cm in fetal neurons. These results indicate that neural cell membrane elasticity decreases with aging.

Membrane elasticity; Aging; Tissue culture; Dorsal root ganglion neuron

1. INTRODUCTION

Recent studies on cultured dorsal root ganglion neurons have revealed that fundamental functions of neural cell membranes change with aging. Neuronal cell membrane affinity to fibronectin and density of fibronectin receptors and membrane fluidity was found to decrease with aging [1–4]. The rate of increase in cell size also decreases with aging after treatment with a hypotonic solution [5]. The increase in cell volume is accompanied by cell membrane expansion. One of the main factors regulating the rate of cell membrane expansion is membrane elasticity. Mechanical properties of the red cell membrane have been studied [6–9]. Since shapes of dissociated DRG neurons appear to be spherical, an elastic area compressibility modulus (EACM) method which has already been used to measure elasticity of erythrocytes [7] is applicable for the measurement of the EACM of neurons. In this study, we applied this method to tissue cultured neurons from a fetal and a 3-month-old mouse to demonstrate that neuronal cell membrane elasticity changes with aging.

2. MATERIALS AND METHODS

Dorsal root ganglia from 20-day-old fetal mice were dissociated in 0.25% trypsin (Sigma) in Ca^{2+}-Mg^{2+}-free Hank's balanced salt solution (HBSS) at 37°C for 15 min. Having been washed 3 times in Ham's F12 containing 10% fetal calf serum (FCS), the cells were further dissociated by gentle pipetting and seeded onto silicon-coated coverslips to prevent the cells from attaching to the coverslips. Dorsal root ganglia from 3-month-old mice were dissociated in 0.25% collagenase in Ham's F12 medium at 37°C for 1.5 h. The dissociated tissues were washed 3 times with HBSS. After another 15 min incubation in 0.25% trypsin in HBSS at 37°C, the cells were dissociated in trypsin inhibitor (Sigma, 0.05 mg/ml) and added to a 30% Percoll (Pharmacia) solution, in which the pH and osmolarity were adjusted. They were then subjected to density gradient centrifugation (10 min, 200 × *g*) to eliminate the myelin sheath. After a double washing in HBSS, the cells were incubated in Ham's F12 containing 10% fetal calf serum and seeded onto silicon-coated coverslips. DRG neurons were identified as having either round or phase-bright cell bodies under a phase contrast light microscope.

The micropipette was mounted on the stage of a Nikon inverted microscope (Nikon, TMD, Japan) and its position was adjusted by a three-dimensional oil pressure micromanipulator (Narishige, MO-303, Japan) operated by remote control. The 1.6 mm glass pipette was pulled to a needle point and then broken by quick fracture to obtain a flat tip in the desired range of 10–17 μm. A capillary with a diameter of a half of a neuron was chosen to suck it up smoothly. After polishing the tip of the pipette by a microgrinder (Narishige, EG6, Japan), the tip was slightly melted in a micro-forge (Narishige, MF-79, Japan) and siliconized to prevent the cells from attaching to the capillary inner wall. The pipette was coupled to a continuous water system supplied by a 16 mm diameter syringe which was fixed on a micromanipulator. A negative pressure in the pipette was produced by raising down a surface level of the water in the syringe by the manipulator.

After 3–8 h in culture, the neurons were sucked into a capillary and the shapes of the neurons were observed with an inverted microscope equipped with a video system (Ikegami, Japan). The magnification on the monitor screen was × 4500. The measurement was carried out at 25°C. As suctioned cells were thought to be rotary bodies, the cell surface areas (A) were calculated from the pictures on the screen. The tension (T) of the membrane was calculated from Laplace's law. EACM values (K) of the neurons were calculated according to the method applied to erythrocytes [7].

3. RESULTS AND DISCUSSION

When the pressure in the capillary was reduced to a more negative level, the neurons were sucked further inside the capillary. When the negative pressure was reduced from −2 mm H_2O to −3 mm H_2O, mature neurons were aspirated further into a capillary. The

Correspondence address: H. Horie, Department of Physiology, School of Medicine, Yokohama City University, 3-9 Fukuura, Kanazawaku, Yokohama 236, Japan

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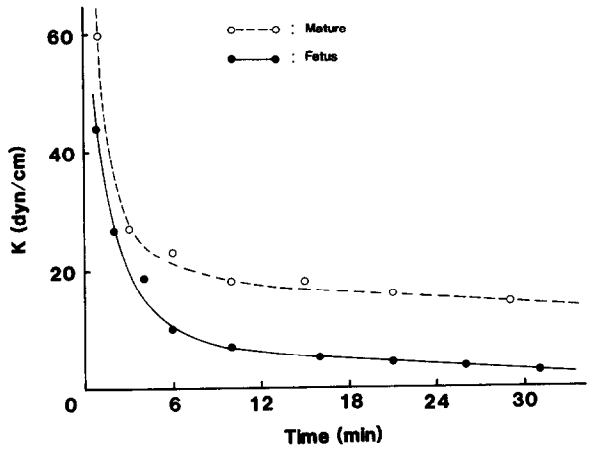

Fig. 1. Time course of changes in the neuronal cell area compressibility (K) after aspiration. (●---●) A 20-day-old fetal mouse DRG neuron; (○—○) 3-month-old mouse DRG neuron.

EACM of the neurons at any time after the change in the pressure were calculated from the picture on the monitor screen. Fig. 1 shows the time courses of the K values of a mature neuron and a fetal neuron, respectively. The K values decreased rapidly and reached a stable point 6 min after the change and then linearly and slowly decayed. The viscoelastic model which was proposed for an erythrocyte membrane includes two components, an elastic component and viscous component [6,9]. According to this model, the initial exponential change in Fig. 1 depends on an elastic component.

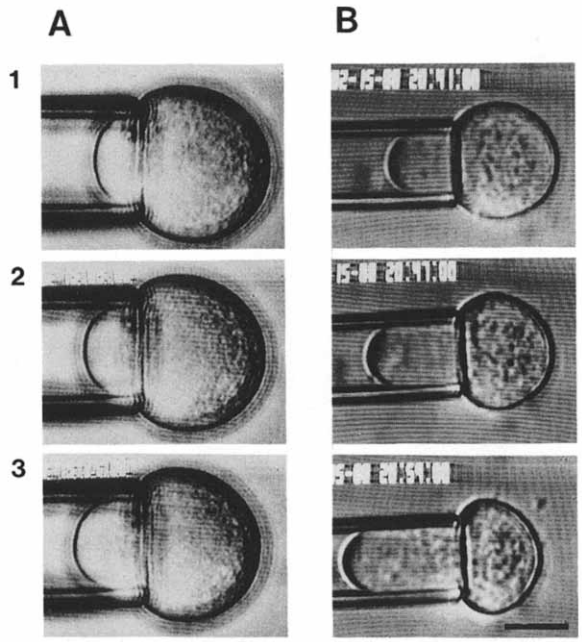

Fig. 2. Measurement of neuronal cell area compressibility from fetal and adult DRG neurons. (A) A 3-month-old mouse DRG neuron. (B) A 20-day-old fetal mouse DRG neuron. The reduction in pressure in a capillary for each sequential step was 1 mm H_2O in the adult mouse and 0.5 mm H_2O in the fetus. Bar = 10 μm.

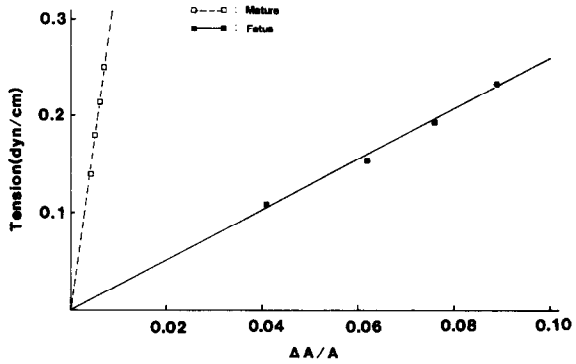

Fig. 3. Neuronal cell area compressibility (K). Plots of the tension vs area expansion calculated from the pictures in Fig. 2 are shown. Two kinds of plots linearly increase and the values of K were 36.5 dyn/cm in adult neurons and 2.66 dyn/cm in fetal neurons, respectively.

From this point of view, the EACM values were measured 6 min after setting the pressure level.

Fig. 2A shows that mature neurons were sucked into the capillary as the negative pressure increased. The lengths of the neurons inside the capillary increased to 5 μm at −2.5 mm H_2O, 6 μm at −3.0 mm H_2O and 6.8 μm at −3.5 mm H_2O. Since a fetal neuron is smaller than a mature neuron, according to Laplace's law a larger negative pressure was applied to a fetal neuron to get a similar membrane tension to that of a mature neuron. Those of fetal neurons inside the capillary increased to 8.3 μm at −4 mm H_2O, 11.4 μm at −5 mm H_2O and 13.2 μm at −6 mm H_2O (Fig. 2B). These results show that a fetal neuron was aspirated further inside the capillary than a mature one. The values of $\Delta A/A$ and ΔT of each neuron were calculated from the pictures in Fig. 2 and plotted in Fig. 3. The ΔT values of each neuron linearly increased as those of $\Delta A/A$ increased. From the slopes of the two lines, the K values were determined to be 36.5 dyn/cm in the mature neurons and 2.66 dyn/cm in the fetal neurons. Fig. 3 shows that the average value of 10 mature neurons was 21.3 ± 10.3 SD dyn/cm and that from 10 fetal neurons was 2.8 ± 0.5 SD dyn/cm. The difference between the two ages of neurons were shown by Student's t-test for $P < 0.05$. Since membrane elasticity was found to be proportional to the inverted value of K, the membrane elasticity of a neuron is thought to decrease with maturation. Since membrane elasticity is

Table I

Difference in the elastic area compressibility modulus (EACM) between an adult neuron and a fetal neuron

Age	EACM (dyn/cm)
Mature	21.3 ± 10.3 (10)
Fetus	2.8 ± 0.5 (10)

The numbers in parentheses show that of the measured neurons. The difference was determined with Student's t-test for $P < 0.05$

related to cell membrane expansion, this result suggests that the cell membrane of a fetal neuron can expand more quickly than that of a mature neuron in response to the treatment with a hypotonic solution. From this suggestion the change in membrane elasticity is a crucial factor causing the rate of initial increase in the diameter after treatment with a hypotonic solution to decrease with aging.

The value of EACM of a mature neuronal membrane is about 1/10 of that of erythrocyte which has a value from 280 dyn/cm to 450 dyn/cm [7]. This result indicates that the neuronal cell membrane is more flexible than the erythrocyte membrane. However, the elastic shear modulus of the membrane of the intact cell and ghost is very close to that calculated for an ideal entropic network of spectrin filaments [10,11]. Spectrin is closely related to the membrane and determines the viscoelastic character of the membrane. In dorsal root ganglion neurons specific networks as in erythrocytes have not been verified, but the intracellular matrix might come in contact with a cell membrane. When colchicine was applied to a neuron, the K value decreased (H. Horie et al., in preparation). This result indicates that microtubules are related to the membrane elasticity in a neuron. Further investigations about the roles of cytoskeletons in membrane elasticity will clarify changes in the membrane elasticity with aging.

REFERENCES

[1] Horie, H. and Kim, S.U. (1984) Brain Res. 294, 178–181.
[2] Horie, H., Kawasaki, Y. and Takenaka, T. (1986) Brain Res. 377, 246–250.
[3] Horie, H., Kawasaki, Y. and Takenaka, T. (1987) Brain Res. 411, 298–303.
[4] Kawasaki, Y., Horie, H. and Takenaka, T. (1986) Brain Res. 397, 185–188.
[5] Horie, H., Ikuta, S., Takenaka, T. and Ito, S. (1989) Brain Res. 477, 233–240.
[6] Evans, E.A. and Hochmuth, R.M. (1976) Biophys. J. 16, 1–11.
[7] Evans, E.A., Waugh, R. and Melnik, L. (1976) Biophys. J. 16, 585–595.
[8] Rand, R.P. and Burton, A.C. (1964) Biophys. J. 4, 115–135.
[9] Rand, R.P. (1964) Biophys. J. 4, 303–316.
[10] Vertessy, B.G. and Steck, T.L. (1989) Biophys. J. 55, 255–262.
[11] Waugh, R.E. and Agre, P. (1988) J. Clin. Invest. 81, 133–141.