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Effect of Intravenously Applied Furosemide on Endocochlear DC Potential and Cochlear Microphonics Correlated with Structural Changes in Guinea Pigs

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ABSTRACT. In the scala media, the DC potential of about +80 mV exists between endolymph and neighboring tissue of the earth potential. Furosemide, which is a strong diuretics, is known to inhibit Na-Cl co-transport across the luminar cell of the thick ascending limb of the Henle loop and it produces the changes in cochlear functions. In this study, furosemide was applied systematically at the dosages of 80 mg/kg, 40 mg/kg and 20 mg/kg. Depending upon the doses, furosemide reduced the DC potential to 0 mV or even negative potential and decreased the amplitude of the cochlear microphonics. These changes started within a few min after application of the drug, were maintained another a few min and then recovered completely to the initial level within an hour or so. It is noteworthy to describe that microphonics recovers faster than DC potential. Electronmicroscopic investigation indicated that the shrinkage and projection took place in the cells of *stria vascularis*. Action of furosemide is explained from the blockage Na-Cl co-transport across the endocochlear side of the marginal cell membrane and, consequently, blockage Na-K pump at the opposite side of the cell membrane.

Key words : endocochlear DC potential — cochlear microphonics — furosemide

The endolymphatic DC potential is about +80mV and is in the opposite direction to the value expected from the K^+ diffusion potential. The positive DC potential is generated by the ouabain sensitive Na-K pump located at the marginal cells of *stria vascularis*.[1)]

Furosemide (FS) is a strong diuretic substance. The diuretic action is mediated by blocking Na-2Cl-K transport or by suppressing K-pump at the thick ascending limb of Henle loop.[2,3)] The ototoxic effects of FS, which are sometimes observed in the course of the treatment of renal failure, are explained by disturbance of the ion transport mechanism across *stria vascularis*, in the same way as in renal tubules.[3,4)]

The present work was carried out to investigate quantitatively the effect of FS on cochlear electrical phenomena.

MATERIALS AND METHODS

Surgical operation: Guinea pigs were anesthetized with pentobarbital (30 mg/kg body weight, i.p.) and were immobilized with succinylcholine chloride. The

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respiration was artificially regulated by a ventilator. After separation of the digastric muscles and removal of styloid processus, the bulla tympanica was opened so widely that the cochlea might be sufficiently observed. A small hole was made on the bony wall of cochlear basal turn. A glass capillary tube filled with 150 mM KCl was inserted through the hole into the cochlear duct and it was advanced by a micromanipulator until EP could be recorded. The body temperature was regulated by a warming pad placed beneath the body. FS was administered intravenously through the jugular vein.

Electro-acoustic equipment: The arrangement of experimental equipment is illustrated in Fig. 1. Sound stimuli were applied from the sound stimulator (DANA JAPAN CONP. DA-502A) through a silicon tube. The stimulating sound was a pip tone of 2500 Hz with a delay time of 2.0 msec and duration of 50 msec.

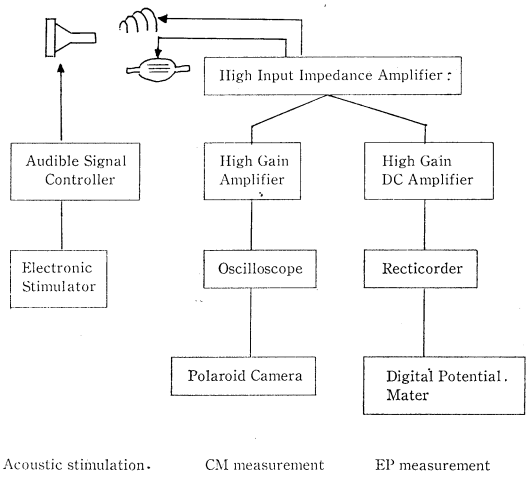

Fig. 1. Block diagram for acoustic stimulation and for recording EP and CM.

Potential recording: The electrode was connected to the high input impedance amplifier and displayed on the cathode ray oscilloscope (VC-10, NIHON KOHDEN) and the pen recorder (RIKA DENKI). Digital potential meter was also used to read EP.

Electronmicroscopic study: The electronmicroscopic changes of the cochlear duct, especially *stria vascularis* were investigated for both control and FS treated animals (30 mg/kg i.p.).

Glutaraldehyde, osmium tetroxide were used for fixation and 70% alcohol was also used for dehydration. The specimens were dehydrated with graded series of ethanol as usual, and were first immersed in 1 : 1 absolute alcohol and styrene for 30 min and then in cold pure styrene overnight. After that they were embedded in small gelatin capsules which were filled with styrene monomer containing 2-3% benzoyl peroxide as catalyst. Polymerization was completed within 24 hr at 60°C, then the specimen was cracked at room temperature using a knife and a hammer. The resin of the cracked pieces was removed from the tissue by dipping in propylene oxide for 2 hr. Specimen was dried

by critical point drying method. The broken surface of the tissue was coated with evaporated goldpalladium and was examined with scanning electron microscope, following the method of Tanaka *et al.*[5]

RESULTS

1. Dose dependence of FS on endocochlear potential (EP) and cochlear microphonics (CM)

When FS was administered intravenously at the dose of 80 mg/kg, EP started to fall first slowly and then very quickly, decreased to the minimum of around −20 mV within 3 min and stayed at the level for about 2 min (Fig. 2).

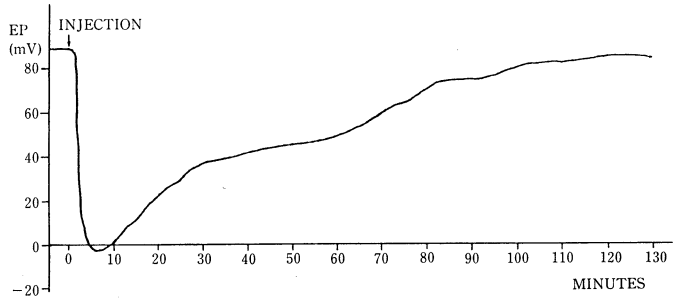

Fig. 2. Time course of the change in EP after injection of FS, 80 mg/kg.

Then, EP recovered very slowly and 100 min was required for complete restoration. Similar time course of decrease in EP was also observed at the dose of 40 mg/kg (Fig. 3). As was expected, the shorter time than the case for 80 mg/kg was enough for complete recovery. The amplitude of CM was also decreased by FS. The peak amplitude of CM was 67.6% of the control at the time when

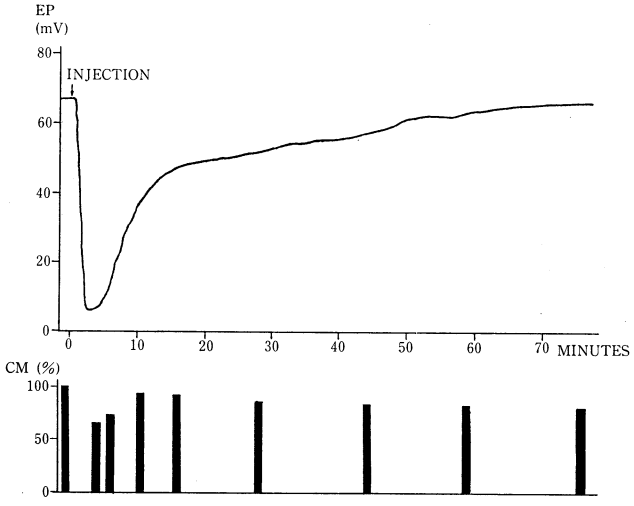

Fig. 3. Time course of the changes in EP and CM after injection of FS, 40 mg/kg.

EP fell to the minimum, but it partially recovered to 73.5% in next 5 min. Although CM started to restore faster than EP, CM did not recover completely to the control even 70 min after FS administration. At the small dose of 20 mg/kg, decrease in EP was less remarkable than those at higher doses (Fig.4). But, the time courses were not so different as the peak amplitudes were.

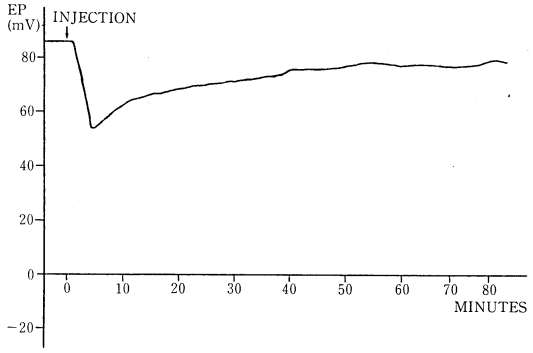

Fig. 4. Time course of changes in EP after injection of FS, 20 mg/kg.

Fig. 5 summarizes the dose dependent decrease of EP. It may be noteworthy to describe first that the main part of the EP fall was very rapid and the time course was independent of the FS doses administered. Secondly, the CM started to recover faster than EP and the time course of recovery of EP and CM was different from each other.

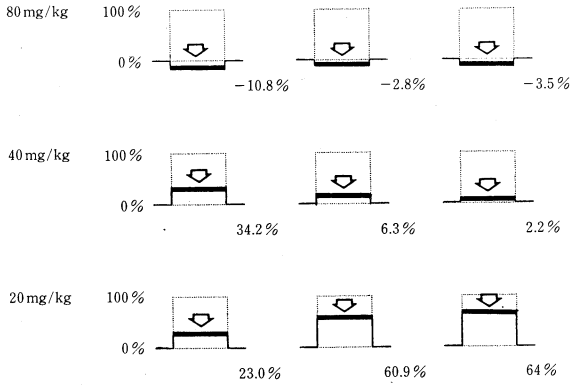

Fig. 5. Change in rate of EP
Peak decrease of EP in 3 preparations of each, dose of FS. Uppermost 3 schemes show peak decrease at 80 mg/kg FS. Attained EP is indicated as the % of control EP before administration of FS. Middle 3 schemes show EP at 40 mg/kg FS. Lowermost 3 schemes at 20 mg/kg.

2. Morphological changes

Marginal cells of *stria vascularis* were projected towards scala media and cellular space was enlarged. We could not find the intermediate cell or basal cell in detail. From these observations FS affects cellular metabolisms of *stria vascularis*. Figs. 5 and 7 show the difference in *stria vascularis* between control and

FS treated one. Nakai *et al.*[6] reported that FS affected enlargement of intracellular space and projection of marginal cell towards scala media, this report was similar to the present findings.

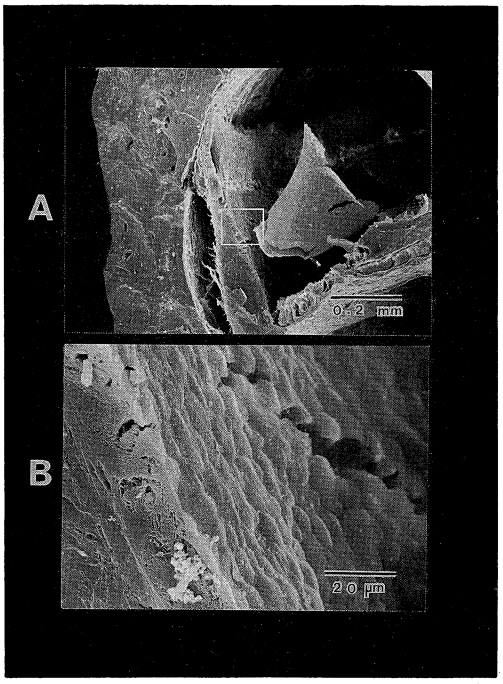

Fig. 6. Control
A ; Scanning electronmicroscopic picture of the marginal cells and cochlear duct.
B ; Marginal cells indicated by squares shown under high magnificate.

DISCUSSION

Several papers have already appeared on the effects of FS on EP or CM.[3,4,7–9] These authors showed that FS decreased EP to 0 mV or even to negative value, dependently on the doses of FS administered. In the present experiment, attention was paid to the dose dependency of the action of FS. EP fell to negative potential if 80 mg/kg FS was administered, but it stayed still at positive value at smaller dose of 40 mg/kg or 20 mg/kg. Although the amplitude of EP was dose dependent, the time course of the change in EP at different FS doses was nearly consistent with each other. The result suggested that the action of FS was in first order Kinetics.

The effect of FS on EP appeared within a minute, and, therefore the effect was the primary action of FS on the *stria vascularis*, not the secondly action due to water loss or diuresis.

The mechanism of action of FS on the cochlear duct must be the same as on the Henle's ascending limb in the kidney. If considered on the basis of the results on renal tubule,[2] Na–K pump is located in the cells of *stria vascularis* at the surface of capillary side and Na-Cl co-transport or Na-2Cl-K co-transport mechanism is located at the endocochlear side. Inflow of Cl^- will produce the

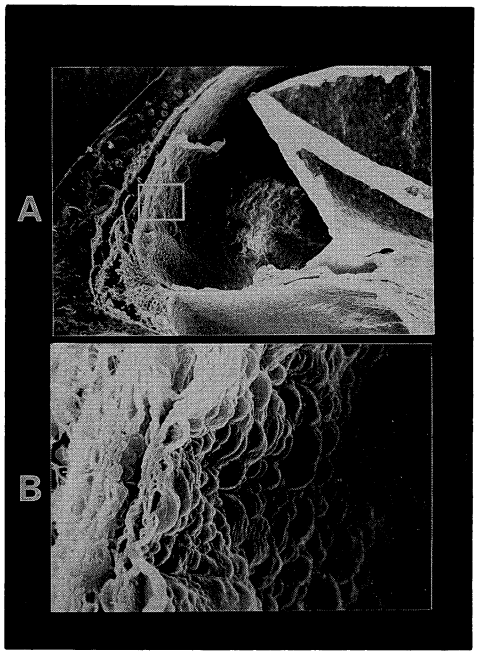

Fig. 7. FS 40 mg/kg
After treatment with 40 mg/kg FS. Projection of the marginal cells towards scala media and enlargement of intercellular space are shown in B.

positive potential of cochlear duct to the cells of *stria vascularis*. FS can block Cl^- transport and consequently turn EP into negative potential. Ouabain or procedure for ischemia also decreases EP, as the results of inhibition of Na-K pump. In this sense, the action of EP is undoubtedly different from that of ouabain.

Recently, ethacrynic acid is reported to decrease the level of intracellular adenylate cyclase.[10,11] It is quite probable that the action of FS is mediated by cAMP. Detailed investigation in renal tubule indicated that FS decreased the cAMP level.[12] The fall in cAMP will inhibit Na-K pump and increase in intracellular Na concentration, which brings the decrease of Cl^- influx. The probable explanation is that FS inhibits Cl^- influx through Na-2Cl-K co-transport mechanism and secondly inhibits Na-K pump by lowering intracellular cAMP level. It is also known that FS inhibits carbonic anhydrase activity. The inhibition of the enzyme activity will produce the accumulation of CO_2 or HCO_3^- in endolymph, which may decrease of EP. However, the concentrations of H^+or HCO_3^- do not affect EP or CM.[13] The action of FS on carbonic anhydrase can be excluded from the possible mechanism on EP or CM.

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