Laser Doppler measurements of inner ear blood flow during experimental thrombosis of cochlear blood vessels in the guinea pig

Abstract This study investigated the vascular effect of ferromagnetic obstruction of cochlear blood vessels in the guinea pig using dual-channel laser Doppler flowmetry. To improve this technique, we tested new types of magnets and iron spheres. In so doing, the cochlear temperature was lowered selectively and general hypothermia was avoided. The success of vascular impairment in the inner ear was found to depend on the experimental conditions used. Given normothermic conditions (38°C body temperature), a clear reduction in cochlear blood flow (CBF) was found in only about 30% of the animals tested when an aluminium-nickel-cobalt alloy magnet and carbonyl iron spheres were used, while this ratio increased to about 80% under general hypothermia (33°C). Using a stronger neodymium-iron-boron magnet and smaller-sized iron spheres, we found the success of vascular obstruction to be approximately 70% under normothermia and 100% with local hypothermia (to 33°C) of the cochlea. Although the extent of vascular impairment revealed a considerable interindividual variation, the present findings demonstrate that ferromagnetic intervention in CBF with dual-channel laser Doppler flowmetry can be used to investigate the effect of quantified cochlear ischemia on inner ear physiology in the guinea pig model and test various therapeutic strategies.

Key words Cochlear ischemia • Ferromagnetic thrombosis • Laser Doppler flowmetry • Guinea pig

Introduction

Although there have been numerous attempts to define the role of impaired cochlear blood flow (CBF) in sudden hearing loss and other inner ear diseases, direct evidence of a vascular etiology is lacking and appropriate animal models are needed for further experimental studies. In previous studies, we tested techniques for local vascular and respiratory intervention in order to produce a definite and stable state of cochlear ischemia [15] and hypoxia [10] in anesthetized guinea pigs. Ferromagnetic obstruction of cochlear blood vessels has been proposed by Giebel et al. [5, 7] as a less traumatic and more selective technique for impairing CBF. The principle of this technique is that cochlear blood vessels are obstructed by the attraction of intravascular iron spheres to an extravascular magnet. However, successful obstruction of cochlear blood vessels was found to occur predominantly under general hypothermia during which body temperature was reduced to 33°C, as shown in our initial [18] and other [6, 20] experiments.

To verify the vascular effect of ferromagnetic cochlear intervention, we continued our methodological studies using dual-channel laser Doppler flowmetry. To improve this technique, we tested new types of magnets and iron spheres in a guinea pig model, and modified the iron suspension injected. We also used local cooling of the cochlea instead of general hypothermia, although opening the tympanic bulla to expose the cochlea results in a substantial decrease in cochlear temperature despite maintaining normal body temperature [8, 13]. Finally, the laser Doppler signals were used to determine whether decreased CBF was due to arterial or venous causes. In a parallel study, we also examined the effect of ferromagnetic obstruction of cochlear blood vessels on the oxygenation and hearing function of the inner ear [19].

Materials and methods

Animal preparations

The animals and physiological procedures used have already been described in detail [16]. Briefly, a total of 62 fasted, healthy adult pigmented outbred guinea pigs (220–600 g body weight) of either sex with normal Preyer's reflexes were used. Animals were anesthetized with ethyl urethane (1.5 g/kg i.p.) and tracheotomized to assist spontaneous respiration. Body temperature was kept at either 38°C or 33°C with a rectal thermistor probe and a thermostatically
controlled heating blanket. In some animals, the temperature of the cochlea was locally lowered to 33°C by means of an anatomically shaped cryoprobe placed in the animals’ oral cavity and perfused with tap water at 17°C. Local temperature was measured on the basal part of the cochlea using a nickel-chromium vs nickel thermocouple (OD 200 μm) [3, 9]. Either the left or right carotid artery was cannulated to inject iron particle suspensions during subsequent experiments. The heart rate was recorded continuously.

Animals were restrained in a supine position with the head fixed in a moveable headholder fastened to a stereotaxic instrument (Narishige, Tokyo, Japan) to position probes employed on the cochlea. To expose both cochleas, a submandibular approach was used [15]. The mucosa and periosteum overlying the cochlea were gently removed, and care was taken to keep the bone dry. Probes were then positioned on the cochlea.

All studies were performed in accordance with the German Prevention of Cruelty to Animals Act and the permission of the Berlin Senate Office for Health (TVV 21/91).

Laser Doppler flowmetry

CBF was measured with a MBF3D dual-channel laser Doppler blood flow monitor (Moor Instruments, Axminster, England). CBF/flux was measured in all animals investigated. In some animals, the concentration of moving red blood cells and their mean velocity were recorded simultaneously. P3L needle probes were used (OD 1.5 mm; fiber separation, 300 μm) and were placed over the lateral wall of the basal or apical parts of the cochlea by means of a micromanipulator, leaving a probe-to-bone distance of about 300 μm [17]. The operating principle of the MBF3D flowmeter has already been described elsewhere [2].

Ferromagnetic thrombosis

Either a 3 × 15 mm (diameter/length) cylindrical aluminium-nickel-cobalt (AlNiCo 500) magnet, (flux density, B = 112 mT) or a 2 × 10 mm neodymium-iron-boron (NdFeB) magnet (B = 219 mT: NeoDeltaMagnet, IBS Magnet, Berlin, Germany) was placed on the basal part of the cochlea. Then, a 0.5-ml portion of a freshly homogenized dispersion of carbonyl iron powder (BASF, Mannheim, Germany) of either type HFF (225 mg, OD 2.93 ± 1.65 μm) or type 1347 HS (25 mg/100 g body weight, OD 1.61 ± 0.81 μm) in Infukoll M40 (Serum-Werk, Bernburg, Germany) was slowly injected into the carotid artery. An additional 0.5-ml portion was given when the first administration failed to affect the CBF.

Experimental protocol

Forty-eight animals were used to verify the ferromagnetic obstruction of cochlear blood vessels using an AlNiCo 500 magnet and a dispersion of HFF carbonyl iron in Infukoll M40, which was predominantly applied contralaterally to the experimental cochlea. In 10 of these animals, body temperature was maintained at 38°C (normothermia). In the remaining 38 animals, body temperature was kept at 33°C (hypothermia).

Animals were separated into two groups of 28 experimental and 10 control animals, respectively. In the experimental group, on average two 0.5-ml portions of the iron dispersion were given at an interval of about 1 h. In 5 controls, the iron dispersions were administered, but no magnet was placed over the cochlea. The 5 other controls received two 0.5-ml Infukoll portions without iron particles to test the effect of the magnetic probe alone on CBF. Fourteen animals were rewarmed to normal body temperature (38°C) after either two iron dispersions or Infukoll alone had been given.

The NeoDeltaMagnet and type 1347 HS carbonyl iron spheres in reduced and body-weight-related amounts were used in a separate group of 14 animals in order to improve the technique of ferromagnetic obstruction of the cochlear blood vessels. In 7 of the animals, body temperature was maintained at 38°C (normothermia). In 7 other animals, the cochlea was cooled locally to 33°C. In these animals, iron portions were administered via the carotid artery either ipsi- or contralaterally to the manipulated cochlea.

Laser Doppler flux signals were recorded continuously from the apical part of the thrombosed and contralateral cochleas over the entire course of the experiment. In the corresponding basal part of each cochlea, signals were only measured at the start and at the end of the experiment. In the animals in which the cochlea was cooled locally, concentration and velocity signals were also recorded in the same manner (i.e., continuously and discontinuously in the apical and basal parts of the cochlea).

Statistical analysis

The readings of the flowmeter (in arbitrary units) were expressed as a percentage of baseline values (100%). Means and standard deviations (SD) were calculated for all parameters measured. The differences between the individual data were examined for their significance using Student’s t-test, with P < 0.05 defined as the level of significance.

Results

AlNiCo magnet and HFF carbonyl iron

Using the AlNiCo 500 magnet and HFF carbonyl iron spheres, a decrease in CBF was only found in 3 out of 10 animals having normal body temperatures (38°C). These findings remained constant despite repeated iron administration. In contrast, when body temperature was lowered to 33°C before infusing the iron, a substantial flux reduction was measured in 23 of the 28 animals tested. In most cases, two separate applications of the 0.5-ml portions of iron were necessary for effective vascular obstruction.

Figure 1 shows a typical time course for apical CBF, heart rate and body temperature following two iron injections and during rewarming to 38°C body temperature. In the thrombosed cochlea (lower curve), CBF dropped by about 25% after the first administration of iron and by an additional 45% after the second iron application. This decrease in CBF persisted when animals were rewarmed to 38°C, even with increased heart rates. In the contralateral cochlea, there was a transient increase in CBF in response to the two injections of the iron suspensions, but this normalized during the post-injection period. No vascular temperature effect was observed during the period of rewarming.

The mean CBF measured in the thrombosed cochleas and controls during hypothermia is shown in Table 1. There was a significant vascular impairment both in the apical and basal parts of the thrombosed cochleas as compared to the controls. The apical flux signal of the thrombosed cochleas amounted to 45% of the initial value and was significantly more reduced than the basal signal, which was decreased to 65% 2 h after the first administration of iron. Because the flux signals of the contralateral ears and controls did not differ significantly, they were grouped together as controls. There was no significant change in their mean values as measured at the start and end of the experiments.