ENDONEURIAL FLUID PRESSURE IN THE FACIAL NERVE OF GUINEA PIGS AND RABBITS

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ABSTRACT

Endoneurial fluid pressure (EFP) was measured in the facial nerve in rabbits and guinea pigs using a servo-controlled micropipette system. EFP in the tympanic segment was 5.3 ± 1.2 cm H_2O in rabbits and 4.1 ± 0.9 and 4.2 ± 0.9 cm H_2O in the tympanic and mastoid segment respectively in guinea pigs, while EFP in the sciatic nerve was around 2 cm H_2O. The higher pressure in the facial nerve may be related to the proximo-distal flow of the endoneurial fluid in peripheral nerves.

The endoneurial interstitium of peripheral nerves is a distinctive physiologic compartment bordered by the perineurial tissue and the nerve-blood barrier along the intrinsic vessels. A positive hydrostatic endoneurial fluid pressure is maintained within the endoneurial interstitium by an osmotic gradient of macromolecules and slightly elevated concentrations of electrolytes in the endoneurial fluid relative to the serum,[1] and by the lack of lymph vessels.[2] The functional significance of this positive EFP is not yet fully understood. In the sciatic nerve of the rat, EFP is increased in different kinds of experimental neuropathies together with nerve edema[3] and neural blood flow is decreased in nerves with increased EFP.[2] To date all EFP measurements available in the literature were performed in the sciatic nerve of the rat and mouse.[2,3,4,5]

The motor fibers of the facial nerve are supposed to be very sensitive to an increased EFP (e.g., in Bell's palsy), probably due to the long course of this nerve through a narrow bony canal. The techniques previously used on the sciatic nerve to record EFP were therefore applied on the facial nerve. The aim of this study is to provide normal data of EFP in the intratemporal portion of the facial nerve in experimental animals.

MATERIAL AND METHODS

EFP was measured with a servo-nulling micropipette system[6] previously used in our laboratory for pressure recording in the inner ear fluids.[7] The principles of the system are explained in Figure 1A. A new, simple calibration procedure was developed to check the proper working of the system immediately before and after the measurements. The part of the pressure recording device containing the pump and the pressure transducer was fixed on a small movable platform. The atmospheric pressure recorded in a drop of Ringer's solution on the surface of the nerve was taken as a reference (0 cm H_2O). Then an additional pressure of +2 cm H_2O (19.6 Pascal) was applied to the fluid in the pipette at the pump side by elevating the pressure transducer and the pump 2 cm relative to the tip of the pipette. For the system this is equivalent to a pressure decrease of 2 cm H_2O in the fluid under investigation (Fig. 1B). Only if such a decrease was plotted, could the pipette be considered to work properly.

Experimental Animals

Rats could not be used because the anatomic situation in the temporal bone is too small. Rabbits were chosen because in this species parts of the tympanic segment of the facial nerve are not covered by bone and thus easily accessible for pressure recording. A second series of experiments were performed in guinea pigs to see if EFP is dependent on animal size.

Seven adult albino New Zealand rabbits were anesthetized with ketamine and xylazine (Ketalar and Rompun), intubated, curarized with alcuronium chloride (Alloferine) and artificially ventilated with N_2O/O_2 and halothane. The tympanic segment of the facial nerve was exposed. Using an

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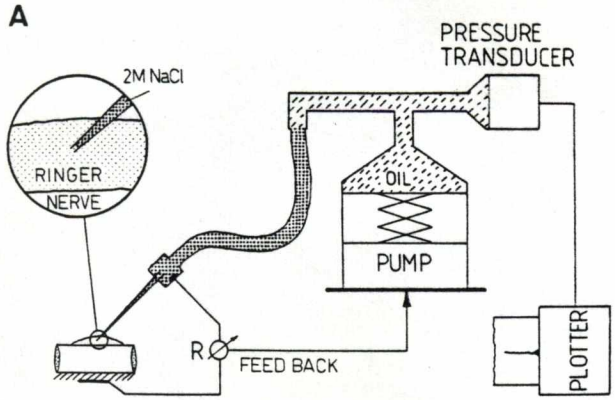

Figure 1A. Principle of the servo-nulling micropipette system. The electrical resistance (R) at an electrical loop between soft tissue and a micropipette filled with a 2 molar sodium chloride solution and introduced into the compartment under investigation is measured. When the pressure in this compartment increases, lower osmolar fluid penetrates into the micropipette and the resistance starts to increase. The resistance, however, is kept constant by a feedback loop to a pump which pushes the lower molar solution out of the pipette tip. The pressure generated by the pump to keep the interface between high and low molar solution at a definite place in the pipette tip equals the pressure in the compartment under investigation and can easily be recorded by a conventional pressure transducer and written out on a plotter. In the pump and pressure transducer the sodium chloride solution is replaced by oil for electrical shielding.

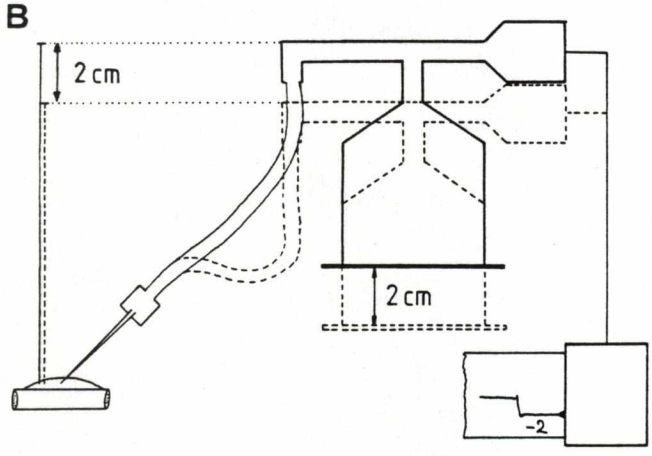

Figure 1B. Calibration procedure. Elevating the pump and the pressure transducer on a movable platform 2 cm relative to the pipette results in a pressure increase of 2 cm H_2O in the pipette on the pump side, which equals a decrease in pressure of 2 cm H_2O in the fluid under investigation. This pressure decrease is written out on the plotter.

operating microscope and a micromanipulator a glass micropipette with a bevelled tip of about 10 μm outer diameter connected to the pressure recording device was first guided into a small drop of Ringer's solution covering the nerve and a calibration was performed as described above. The pipette was then advanced about 0.5 mm through the epineurium into the nerve and carefully withdrawn until the initial deformation of the nerve surface disappeared and stable pressure was recorded. Usually a clear "jump" of the pipette through the epineurium could be observed. At each puncture the pipette was left in situ for 20 to 40 seconds. Respiratory movements which lead to large artifacts in the pressure curves were abolished by stopping the ventilation as long as the pipette was in the nerve. After several penetrations the animals were sacrificed. The facial nerves of three animals were processed for light microscopy and examined for traces of penetrations of the micropipettes. To get more information on the pipette tip localization additional experiments were performed with punctions of the nerve with the same pipettes but filled with Evans blue dye in 0.9 percent saline. After the pipette tip was introduced through the epineurium a minimal amount of the dye was injected through the pipette with a syringe. Frozen sections of these nerves were examined under a fluorescence microscope. Red fluorescent spots of about 150 μm were found 100 μm below the epineurium.

In a second series of experiments, EFP was recorded in the mastoid segment of the facial nerve in 12 pigmented guinea pigs weighing 400 to 700 g. In four of them EFP was recorded also in the tympanic segment, and in four animals also in the sciatic nerve. Attempts to record the EFP in the extratemporal portion of the facial nerves were not successful due to the very strong epineurium distal to the stylomastoid foramen. The guinea pigs were anesthetized with ketamine and xylazine I.M. and pentobarbital (Nembutal) I.P., tracheotomized and mechanically ventilated with room air. The facial nerve was exposed at the stylomastoid foramen, and the bulla and the posterior wall of the mastoid segment of the facial nerve canal was carefully opened with a diamond burr leaving the epineurium intact. The tympanic segment was reached through the epitympanum and the Fallopian canal was opened over 2 mm between stapes and the tensor tympani muscle. Respiratory artifacts were not as large as in the rabbit, thus the ventilation was only interrupted during short periods when the pipette was in the nerve.

RESULTS

1. EFP in the Tympanic Segment of the Facial Nerve in Rabbits

Figure 2 shows two representative pressure recordings in the tympanic segment of the facial nerve in a rabbit. Before the respirator was stopped oscillations of the pressure curve were already seen when the pipette was on the nerve surface in a drop of Ringer's solution. When the pipette penetrated the intact epineurium the pressure rose over 10 cm H_2O but then immediately stabilized around 7 cm H_2O with small (about

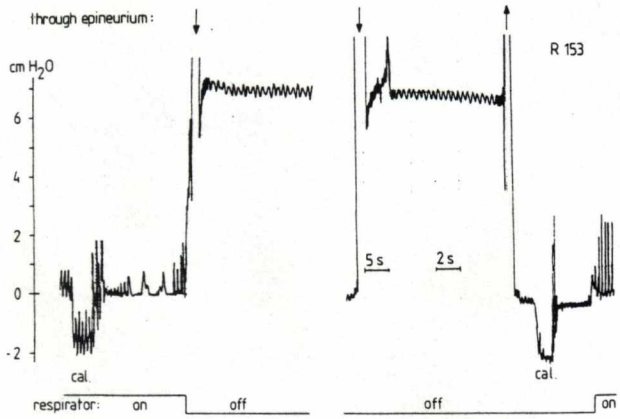

Figure 2. Two representative pressure recordings from the tympanic segment of the facial nerve in a rabbit. Before and after punction of the nerve a calibration (cal.) of −2 cm H_2O is performed on the surface of the nerve. Oscillations induced by respiratory movements are seen when the pipette tip is in Ringer's solution on the nerve. The respirator is stopped immediately before the pipette penetrates the epineurium. The pressure increases to over 10 cm H_2O but then stabilizes and smaller pulsatory oscillations become visible.

0.3 cm H_2O) pulsatory oscillations. In each animal two to five recordings were performed. Sometimes quite large variations were observed between single recordings (Table 1), but with exception of animal No. 72 the mean pressure did not differ much between the different animals.

Table 1. Endoneurial Fluid Pressure (EFP) in the Tympanic Segment of the Facial Nerve in Seven Rabbits

Animal No.	*EFP in cm H_2O (single recordings)*	*Mean EFP ± 1 SD*
161	4.2	5.0 ± 1.1
	5.8	
154	4.0	5.8 ± 2.4
	5.0	
	8.5	
153	3.5	6.1 ± 1.5
	6.0	
	7.0	
	7.0	
	7.0	
151	3.5	4.3 ± 1.4
	3.5	
	6.0	
130	6.0	6.5 ± 0.7
	7.0	
90	3.5	6.5 ± 2.6
	7.5	
	8.5	
72	2.0	3.2 ± 1.3
	2.5	
	2.5	
	3.5	
	5.5	
Mean		5.3 ± 1.2

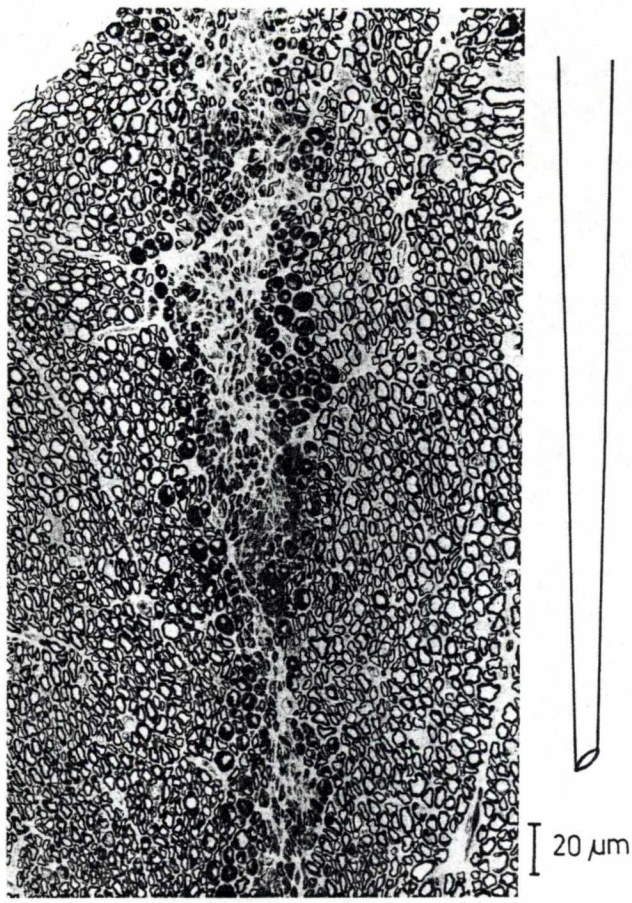

Figure 3. Photomicrograph of a tympanic segment of the facial nerve in a rabbit showing a penetration path of a micropipette. The shape of a micropipette is drawn scematically with the same magnification for comparison.

Histologic examinations of three nerves revealed traces of pipette penetrations with moderately damaged nerve fibers along a 30 to 100 µm wide trace about 0.5 mm into the nerve (Fig. 3).

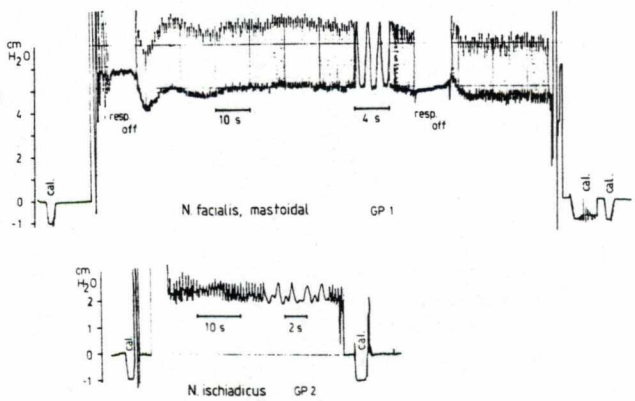

Figure 4. EFP recordings from the mastoid segment of the facial nerve and from the sciatic nerve in guinea pigs. Recordings were made with two different paper speeds to show both respiratory and pulsatory pressure oscillations. (For legends see also Fig. 2.)

Table 2. EFP (cm H_2O) in the Facial and Sciatic Nerve of 12 Guinea Pigs

Animal No.	EFP mastoid segment mean ± 1SD	N	EFP tympanic segment mean ± 1SD	N	EFP sciatic nerve mean ± 1SD	N
1	4.9 ± 1.4	4	4.4 ± 0.4	5	2.1 ± 0.4	3
2	3.8	1	4.1 ± 1.2	3	1.7 ± 0.4	5
3	5.1 ± 0.3	4	5.0 ± 1.4	2	n.d.	
4	2.8 ± 0	2	2.8 ± 0.4	2	n.d.	
5	3.7 ± 0.3	6	n.d.		2.3 ± 0.4	2
6	2.8 ± 0.4	2	n.d.		1.4 ± 0.1	3
7	3.7 ± 0.7	3	n.d.		n.d.	
8	5.5 ± 0.4	4	n.d.		n.d.	
9	3.8 ± 0	2	n.d.		n.d.	
10	4.2 ± 0.8	4	n.d.		n.d.	
11	5.2 ± 0.8	3	n.d.		n.d.	
12	4.6 ± 0.4	5	n.d.		n.d.	
mean	4.2 ± 0.9		4.1 ± 0.9		1.9 ± 0.4	

N = number of successful measurements in each nerve
n.d. = not done

2. EFP in Guinea Pigs

Figure 4 compares pressure recording curves from the facial nerve and from the sciatic nerve in the guinea pig. In contrast to the recordings in rabbits these curves were written without interruption of the artificial ventilation. The mean EFP in the mastoidal segment was 4.2 ± 0.9 cm H_2O (12 animals) and 4.1 ± 0.9 in the tympanic segment (4 animals) with no significant difference between these recording sites (Table 2). The EFP in the sciatic nerve, however, was lower (1.9 ± 0.4).

DISCUSSION

The servo-controlled micropipette system proved to be a practical tool for measuring endoneurial fluid pressure in the intratemporal portion of the facial nerve in rabbits and guinea pigs, although it was technically much more difficult to record the pressure in the endoneurial interstitium which is a virtual fluid space than in a definite fluid space as, for example, in the scala tympani or scala media of the inner ear. The main problems were blocking of the electrode tip by tissue or breaking of electrodes during penetration of the epineurium. Therefore several penetrations in the same nerve gave variable pressure values, which had to be averaged. This was reported also in the sciatic nerve of the rat and interpreted as different pressures in different fascicles.[4] In the facial nerve, however, there are no distinct fascicles. The EFP in the sciatic nerve in guinea pigs (1.9 cm H_2O) agrees well with EFP reported in the same nerve in rats.[2,3]

The EFP in the tympanic segment of the facial nerve was 5.3 ± 1.2 cm H_2O in rabbits and 4.1 ± 0.9 and 4.2 ± 0.9 in the tympanic and mastoid segment respectively in guinea pigs. The slightly higher EFP in rabbits may be due to the larger size of this species in which other physiologic parameters are also higher. Arterial blood pressure, for example, is 110/80 in rabbits but 90/56 mm Hg in guinea pigs.[8] EFP in the sciatic nerve of the mouse is also lower (1.6 ± 0.5) than in the rat (2.0 ± 1.0).[5] In the intratemporal portions of the facial nerve, EFP is about twice as high as in the sciatic nerve. There were differences in the surgical approach to the nerves which might have influenced the EFP measurement: the sciatic nerve is easily accessible without traumatizing the nerve, while exposing the intratemporal facial nerve requires drilling on the bony facial nerve canal. In crush injuries of the sciatic nerve, elevation of EFP required 90 minutes to develop.[9] Our pressure recordings were performed immediately after opening the bony canal and in the rabbits no drilling over the nerve was necessary. The higher EFP in the facial nerve is probably due at least in part to the recording site being more proximal to the central nervous system. Very recently, EFP in the facial nerve of guinea pigs was reported to be directly related to cerebrospinal fluid pressure.[10] Endoneurial fluid moves by simple bulk flow in a proximo-distal direction and escapes from the endoneurium at the most distal end in the nerve[3] where there is negative pressure in the interstitial tissue.[11] However, no pressure gradient between the tympanic and the more distal segment was found in our guinea pigs. Unfortunately, attempts to record EFP in the extratemporal portions of the facial nerve were not successful.

In a preliminary study[12] we have found a 60% elevated EFP in the tympanic segment of the facial nerve 3 months after nerve transplantations at the stylomastoid foramen. Further experiments will be necessary to understand the unique reaction of EFP in the facial nerve to injury and compression and its clinical implications.

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