Cochlear implantation, a medical intervention utilizing electrical field stimulation

University Doctoral Thesis

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SZEGED

1997

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Content

Background

Cochlear implantation (CI) has been established world-wide as a safe and effective method of rehabilitation of profoundly hearing-impaired adults and children, who derive insufficient benefit from simple sound amplification (Sommerfield & Marshall, 1995). A cochlear implant is an electronic device that is inserted into the inner ear of a totally deaf person to introduce or restore the perception of sound. An external detectable component comprises a microphone, a small battery powered device for processing the signal, and an induction coil that transmits the refined signal through the skin to the implant. The notion that an electric current applied to the cochlea or to the auditory nerve might be perceived by the brain as sound arose as early as 200 years ago. However, the idea became a clinical reality only in 1957, when Djourno and Eyries implanted a rudimentary single channel electrode into the ear of a patient who was having surgery to eradicate a cholesteatoma in his only hearing ear (Djourno & Eyries, 1957). By stimulating the electrode with an external current generator they were able to produce a perception of sound that the patient likened to crickets chirping. Then cochlear implant technology evolved rapidly, and reliable commercially manufactured devices have been available since the early 1980s. Otologists, especially those in Great Britain, initially viewed the technique with some scepticism as to whether such a relatively modest apparatus could replicate the complex physiology of the human cochlea (Brimacombe et al., 1994; Haggard, 1991). Nevertheless, studies from North America, Australia, and Europe have now dispelled doubts about the effectiveness of cochlear implants in several groups of totally deaf people (Ganz et al., 1994). Moreover, detailed analyses have been provided on both results and complications of this medical intervention (Cohen et al., 1988; Cohen & Hoffmann, 1993; El Naggar & Hawthorne, 1995).

The currently used intracochlear implants work with multiple electrodes in their electrode array. The system functions in the way as follows: an external microphone picks up sound waves and sends them through wire to the speech processor. The speech processor breaks down the sounds and codes them, then sends them back up the wire to an external transmitter. The transmitter emits a radio signal which is picked up through the skin by the implanted receiver. The receiver/stimulator sends the appropriate electrical signals to the

electrodes in the cochlea. The electrodes stimulate the neural elements there. The hearing nerve carries the resulting nerve impulses to the brain, which interprets them as sounds. As the electrodes of the array are not in mechanical contact with neural elements of the ggl. spirale, we have proposed that function of the electrode array to evoke neural activation seems to be similar to that seen with electrical field stimulation, a convenient method in physiology to study the effect of neurotransmission on various 'end organs'(Szilvassy et al., 1995 and 1997). The aim of our work was therefore to study baseline mechanisms activating during electrical field stimulation of the cochlea (experimental study) as well as to get an insight into cochlear implantation as a clinical example of electrical field stimulation 'in situ'.

Part I (Experimental work)

In this chapter, we describe an original method developed in 1995 to study the possible effect of electrical activation of the cochlea on vascular tone.

Vascular effects of electrical activation of the cochlea

Introduction

The electrically stimulated cochlea has been shown to release neurotransmitters such as acetylcholine, calcitonin gene-related peptide (CGRP), nitric oxide (NO) and glutamate playing both mediatory and modulatory role in cochlear function (Chu et al., 1995; Erostegui et al., 1994; Plinkert et al., 1991; Tohyama et al., 1989),. Since these neurotransmitters are known to alter vascular tone (Moncada et al., 1991; Lazdunski et al., 1994), we postulated that at least under experimental conditions, electrical activation of the cochlea can be reflected in changes in vascular tone.

Methods

Electrical stimulation of isolated cochlea of the guinea pig

Cochleas were prepared from pentobarbitone- (40 mg/kg)-anaesthetized healthy adult guinea pigs and placed into an organ chamber containing Krebs bicarbonate buffer solution maintained at 37°C. Each 5 ml solution contained 118.1 mM NaCl, 4.7 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11.1 mM glucose. Solution was aerated with 95% O_2 and 5% CO_2 and kept at a pH of 7.4 ± 0.05.

To release neurotransmitters from the cochlea, field stimulation (FS) utilized 50 Hz, 80 V square impulses of 0.2 ms duration applied by means of an "EXPERIMETRIA" (London, England) programmable stimulator as described (Lonovics et al., 1994; Szilvassy et al., 1996).

Characterization of the vascular effects of electrical activation of the cochleas prepared

In order to define the vascular effects of the neurotransmitters, 4 mm long aortic rings were prepared from healthy male New Zealand rabbits weighing 3000-3500 g and tested for changes in isometric tension as described (Szilvassy et al., 1995).

The rings were allowed to equilibrate over 1 h at an initial tension of 20 millinewtons (mN). The preparations were then exposed to cumulative increases in phenylephrine concentrations followed by cumulative increases in acetylcholine concentration to test functional integrity of vascular endothelium (Furchgott & Zawadski, 1980). After washout, the rings were precontracted with 1 μM phenylephrine and were then exposed to the organ fluid of the cochleas in which the field stimulation had been accomplished.

Experiments were repeated with aortic rings having functionally intact endothelium and those from which the intimal surfaces had been removed. Rings with intact endothelium were tested for relaxation responses to the "cochlear fluid" in the presence of 30 μM N^G-nitro-L-arginine methyl ester (L-NAME) and successive incubation with 3 mM L-arginine (incubation period: 30 min for each). After washout, phenylephrine-precontracted aortic rings were exposed to the "cochlear fluid" in the presence of cumulative increases of glibenclamide and/or its vehicle concentrations. Finally, after washout, field stimulation was repeated in the presence of 1 μM tetrodotoxin added to the cochleas.

Statistical analysis

All data were expressed as means$\pm$sd and were statistically analyzed by analysis of variance followed by the Bonferroni *t*- test. The level of significance was $p<0.05$.

Results

Changes in isometric tension

Phenylepinephrine-precontracted vessel rings relaxed in response to cochlear FS. Removal of the vascular endothelium blocked the relaxation response. The relaxation

response was blocked by L-NAME when added to the organ chamber of the vascular preparations with intact endothelium. In contrast, L-NAME added to the organ chamber of the cochlea did not influence the FS-induced vasorelaxation. Additional incubation with L-arginine blocked the inhibitory effect of L-NAME (Figure 1). Tetrodotoxin added to the cochlear fluid also blocked the effect of FS (Szilvassy et al., 1997).

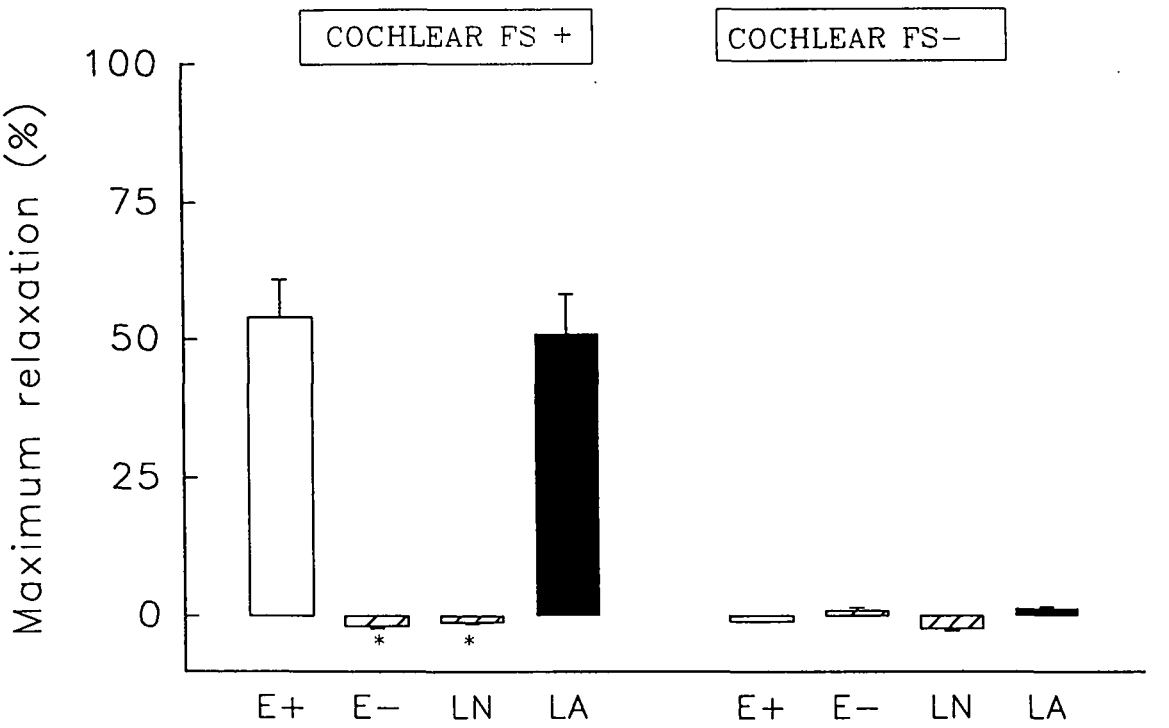


Figure 1. Endothelium dependent vasorelaxation by cochlear fluid. E+:rings with functionally intact endothelium; E-:rings from which the endothelial layer has been gently removed; LN: L-NAME; LA: L-Arginine. The data are means ± S.D. obtained with 6 preparations. *: significantly different from E+ at $p<0.05$.

Changes in vascular cyclic nucleotides

These results suggested that the vasorelaxation response to exposure to organ fluid of the electrically stimulated cochleas were due to an endothelial release of NO. Since the majority of smooth muscle relaxing effect of NO is thought to be mediated by cyclic guanosine 3':5' monophosphate (GMP), we carried out some further experiments to study whether the aforementioned vasorelaxation parallelled an increase in vascular cyclic GMP content.

Changes in vascular cyclic adenosine 3':5' monophosphate (AMP) and cyclic GMP contents in response to exposure to the cochlear fluid at the above protocol were determined in separate rings using Amersham radioimmunoassay (RIA) kits (Les Ulis, France) as described previously (Szilvassy et al., 1994).

Exposure to cochlear fluid after field stimulation significantly increased levels of vascular cyclic GMP and AMP. Baseline values of either cyclic nucleotide did not change when rings were exposed to fluid of the non-stimulated cochleas when field stimulation was performed in the presence of 1 μM tetrodotoxin (TTX) or when the effect of cochlear stimulation was tested on endothelium-free vessel rings (Figure 2a,b).

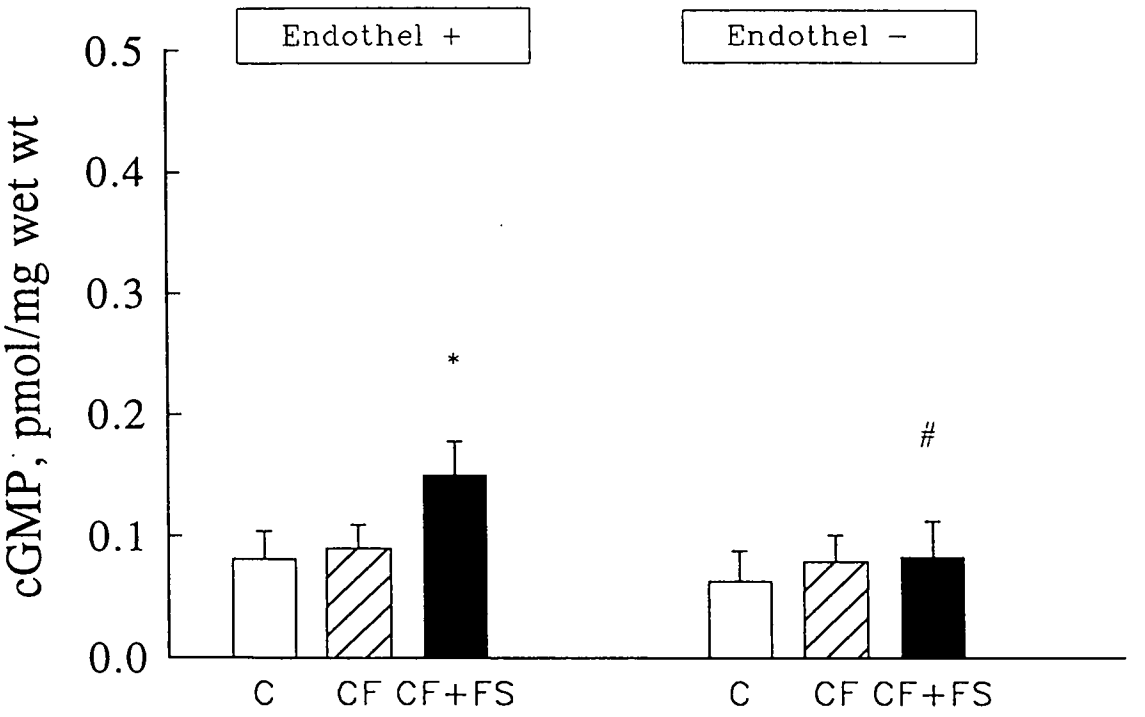


Figure 2a Effect of cochlear fluid on vascular cyclic GMP content. C: control; CF: exposure to cochlear fluid (cochlea is not stimulated) ; CF+FS: exposure to fluid of cochleas exposed to preceding field stimulation. Data are means ± S.D. obtained with 5 preparations. *: different from control at $p < 0.05$; #:endothel- vs endothel+ preparations at $p < 0.05$.

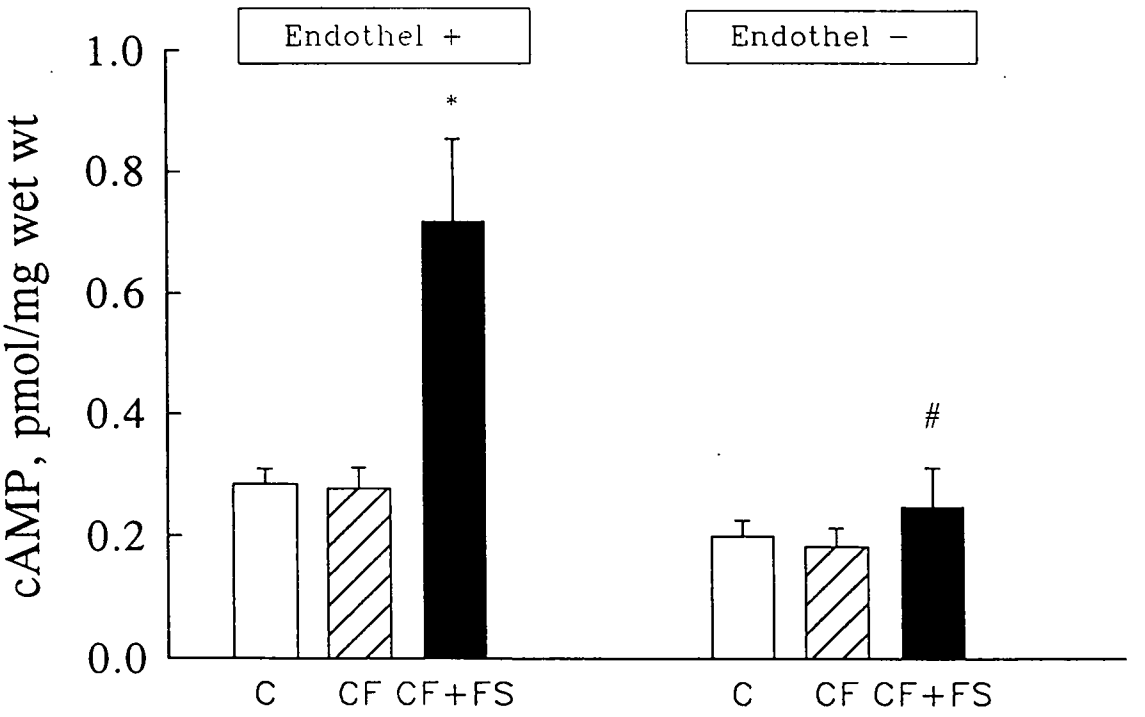


Figure 2b Effect of cochlear fluid on vascular cyclic AMP content. C: control; CF: exposure to cochlear fluid (cochlea is not stimulated) ; CF+FS: exposure to fluid of cochleas exposed to preceding field stimulation. Data are means ± S.D. obtained with 5 preparations. *: different from control at $p < 0.05$; #:endothel- vs endothel+ preparations at $p < 0.05$.

Glibenclamide sensitivity of NO-mediated vasorelaxation by cochlear stimulation

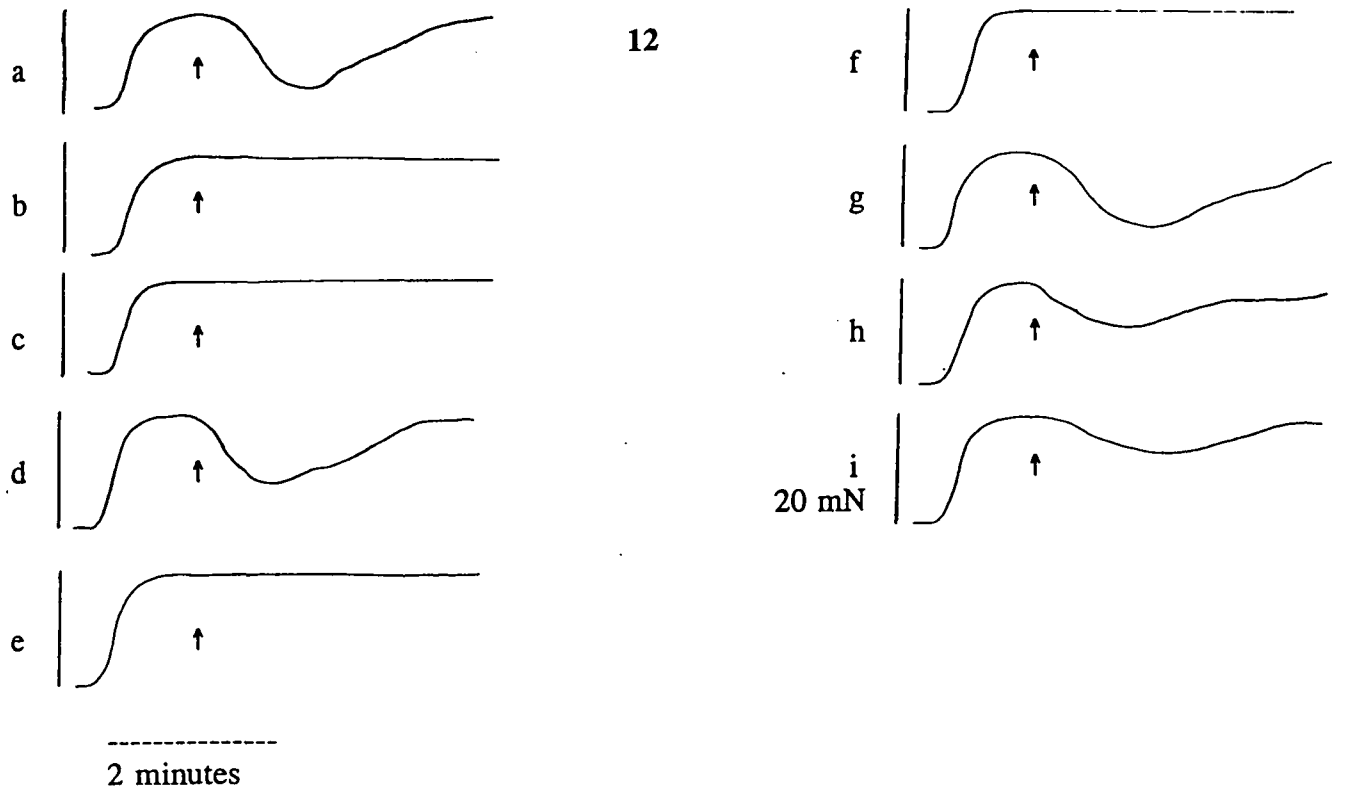
Based on the above results, we concluded that the L-arginine nitric oxide (NO) pathway played a major role in vasorelaxation by cochlear nerve stimulation. Moreover, vascular endothelium could be identified as the site of NO formation. Nitric oxide relaxes smooth muscle cells mainly through stimulation of cyclic GMP formation. Cyclic GMP, however, has been found to activate ATP-sensitive potassium (K_{ATP}) channels in vascular tissue (Kubo et al., 1994; Armstead, 1996). In addition, NO has been proposed to stimulate the release of other neurotransmitters such as vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP). VIP and CGRP, similar to several hormones and neurotransmitters with smooth muscle relaxing effect that share the ability to activate adenylyl cyclase, have been suggested to attain at least some of its effect through opening of K_{ATP} channels [Quayle & Standen, 1994). Therefore, we explored the possibility that K_{ATP} activation was involved in cochlear stimulation-induced vasorelaxation.

Experimental protocol

Aortic rings with intact endothelium were preincubated with glibenclamide concentrations (0.1-10 μM) before exposure to fluid of the stimulated cochleas.

Results

Glibenclamide inhibited cochlear stimulation-induced vasorelaxation in a concentration-dependent manner. Nevertheless, the K_{ATP} channel blocker failed to completely inhibit the relaxation response (Figure 3).



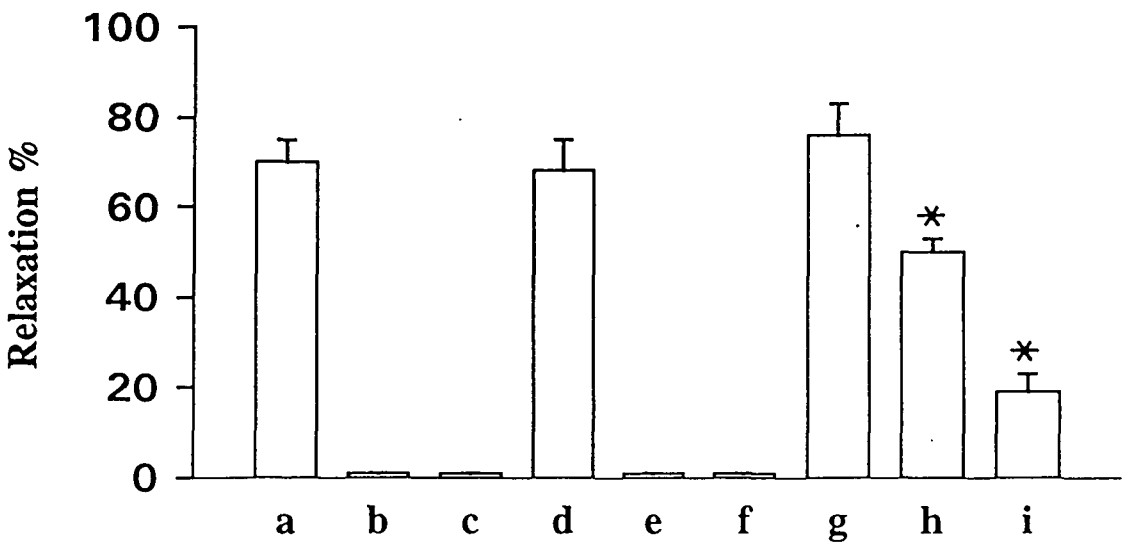


Figure 3. Involvement of K_{ATP} channels in relaxation of aortic rings induced by exposure to 'cochlear fluid'. The arrows indicate the moment of exposure. a:without treatment; b:without field stimulation; c: with L-NAME; d: L-NAME+L-Arg; e: without endothelium; f:TTX to cochlea; g:0.1 μM glibenclamide to rings; 1 μM to rings; i: 10 μM glibenclamide to rings. Data are means$\pm$S.D. at n=6. *: with vs without glibenclamide at $p<0.05$.

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Discussion

The present results indicate that electrical field stimulation of isolated guinea pig cochleas produces relaxation in rabbit aortic rings which is endothelium-dependent. In parallel with the relaxation response, a significant increase was found in both cyclic AMP and GMP contents in the aortic tissue. Relaxation was blocked by L-NAME, an inhibitor of NO synthase. Nevertheless, this inhibitory effect was reversed by additional incubation with L-arginine, indicating the relaxation response to be essentially nitrergic.

Glibenclamide, a specific blocker of K_{ATP} channels was found to attenuate the cochlear stimulation-induced relaxation of the rabbit aortic rings in a concentration-dependent manner, suggesting that K_{ATP} channel activation was involved in the relaxation mechanism. Since tetrodotoxin added to the cochlear preparation also blocked both the vascular response and changes in cyclic nucleotide content, our findings suggest that the currently undefined substance(s) from the electrically stimulated cochlea producing vasodilation are of neural origin.

Several neurotransmitters and neurohormones have been reported to exert at least some of their biological effect through activation of K_{ATP} channels. These include galanin, vasoactive intestinal polypeptide, CGRP, prostacyclin and norepinephrine . These substances, however, share an ability to increase intracellular cyclic AMP level in target cells. An increase in cyclic AMP has been shown to be followed by K_{ATP} channel opening involving protein kinase A (Lazdunski, 1994). Nevertheless, any cyclic AMP elevating agent released from the cochlea could hardly be responsible for the vasorelaxation observed in our experiments, since such an agent would be expected to preserve its vasodilatory effect in the absence of an endothelial layer.

Many agents relax arterial smooth muscle through stimulation of the release of inhibitory factors from endothelial cells (Furchgott & Zawadski, 1980). At least two endothelial factors are known to exert relaxation: NO, which was previously described as endothelium derived

relaxing factor (EDRF) Moncada et al., 1991), and endothelium derived hyperpolarizing factor (EDHF, Taylor & Weston, 1988). Both EDRF and EDHF can elicit hyperpolarization in vascular smooth muscle cells with the involvement of K_{ATP} channels. In our study, glibenclamide was found to attenuate endothelium-dependent vasorelaxation by our presumed cochlear neurotransmitters in a concentration-dependent manner. Nevertheless, since NO synthase inhibition completely blocked the relaxation response, we believe that NO-induced relaxation is only partially mediated by K_{ATP} channel activation.

It is widely accepted that activation of smooth muscle soluble guanylate cyclase by NO results in the accumulation of intracellular cyclic GMP, and produces a sequence of protein phosphorylation that leads to relaxation Moncada et al., 1991). An increase in cyclic GMP levels might result in an increase in cyclic AMP to promote relaxation through inhibition of cyclic AMP breakdown by interaction between cyclic nucleotide phosphodiesterases. K_{ATP} channel opening seems to be profoundly involved in this mechanism, either due to a secondary increase in cyclic AMP, or by a direct cyclic GMP-regulated pathway. However, the lack of complete inhibition of cochlear stimulation-induced vasorelaxation by glibenclamide suggests either an incomplete blockade of these channels or the presence of an additional, K_{ATP} -independent relaxation mechanism by NO. Whatever the precise mechanism is, our present results seem to support the concept that the endothelial layer is a transducer of important vascular signals to which the vascular bed must respond in order to ensure adequate tissue perfusion according to moment-to-moment requirements. Our results also imply a possible beneficial effect of pharmacologic maneuvers that utilize the NO→cyclic GMP pathway to support the clinical use of cochlear electrical stimulation.

Part II (Clinical study)

In this chapter, we describe intracochlear implantation as a clinically applied field stimulation method.

Electrical stimulation of the cochlea

Interest in the electrical stimulation of the cochlea has a long history, beginning with Alessandro Volta's experiments in 1800, in which he stimulated his own ears electrically. The stimulation itself did not prove to be a pleasant experience but produced some sound sensation. In 1930, Weaver & Bray discovered that the cochlea essentially acts as a transducer of acoustic energy, which is then transmitted via the auditory neural pathway to the brain. This provided further inspiration for the possibility of evoking artificial hearing through direct electrical stimulation. Advances in cardiac pacemakers in the 1950s and 1960s increased knowledge of biocompatible materials, insulation of electrodes and the effects of electrical stimulation which helped advances in cochlear implant research. The implanted electrodes to which the computed electrical signals are transferred serve as final effectors of the implant system. Cochlear implants have been described as monopolar or bipolar according to the relative positions of the active and reference electrodes. In a monopolar system, the active and reference electrodes are positioned remotely, e.g. the active electrode may be inside the cochlea and the reference electrode embedded in muscle tissue outside the cochlea. If the active and reference electrodes are in close proximity, e.g. adjacent to each other inside the cochlea, the implant is known as a bipolar system (Figure 4**ab**). Whatever the electrode insertion setting is, the electrical current that flows from an active electrode (or positive pole) to a reference electrode (negative pole) induces an *electrical field* to stimulate nerves *around* the electrodes. Thus, cochlear implantation can be referred to as the application of electrical field stimulation in clinical setting. Nevertheless, electrical field stimulation stimulates all kind of neurons in the corresponding electrical field with minimum or no selectivity for neural elements intended to excite. Therefore, it is not surprising that the use of cochlear implants sometimes results in adverse effects such as eye twitching, painful sensations and/or facial twitching (Muller-Deile et al., 1994!!). An example of facial twitching due to inappropriate electrode positioning is indicated by Figure 5.

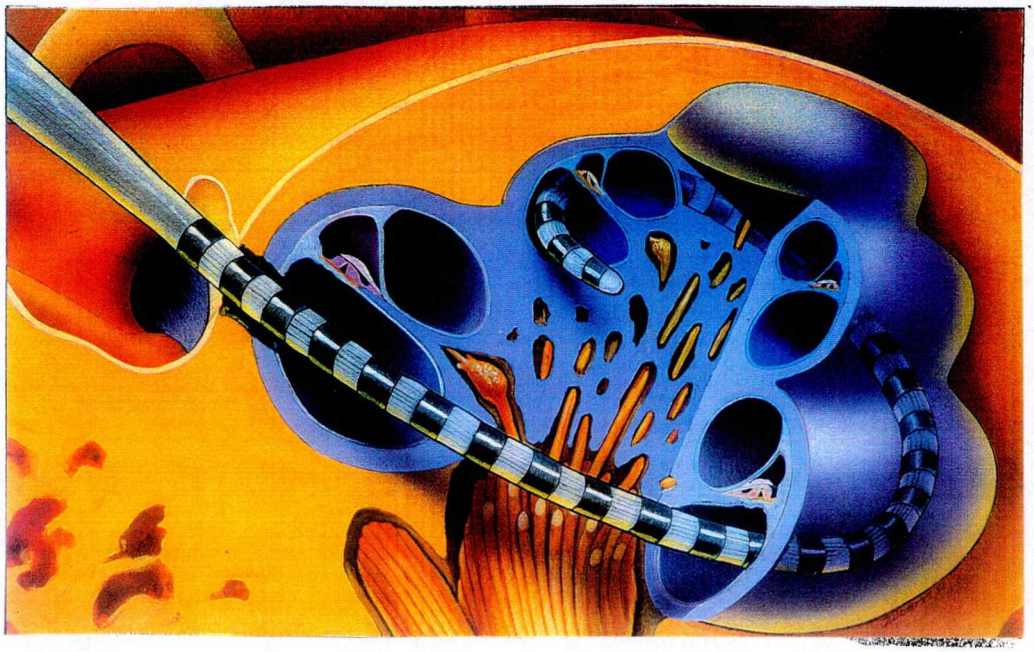


Figure 4a. Schematic representation of multichannel electrode positioning in the scala tympany.

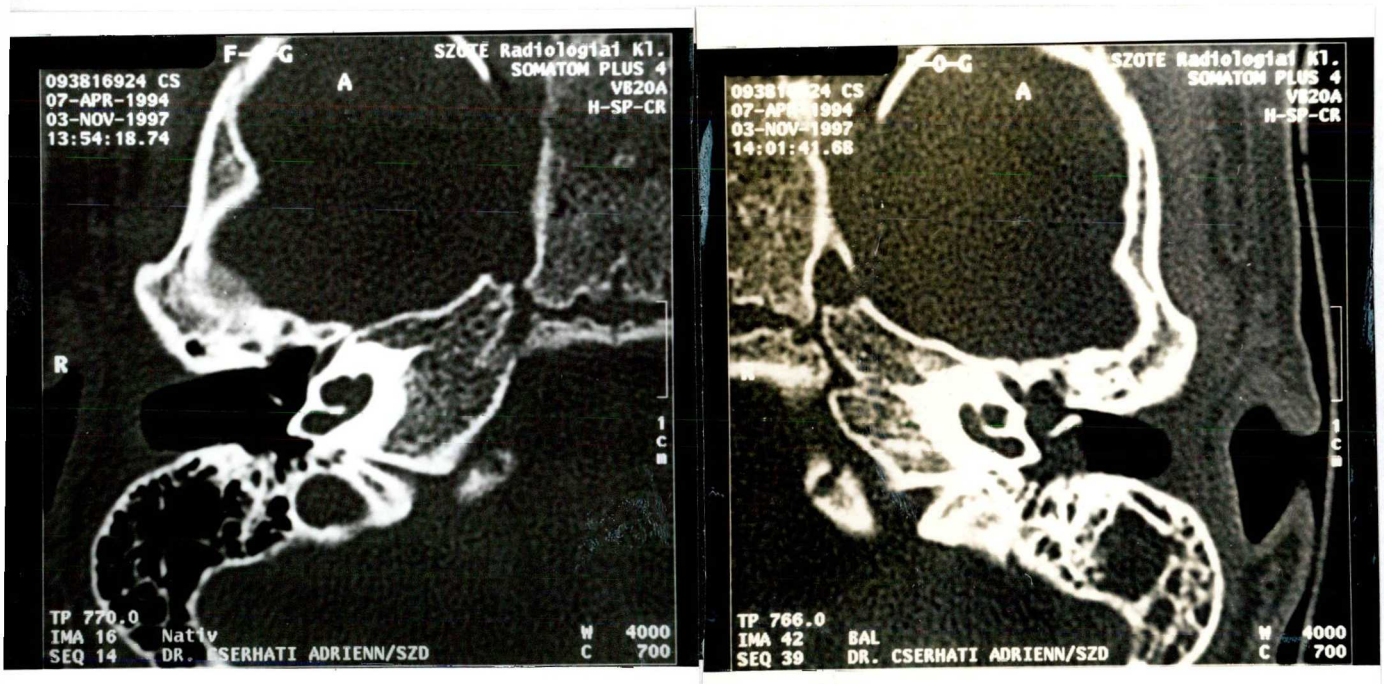
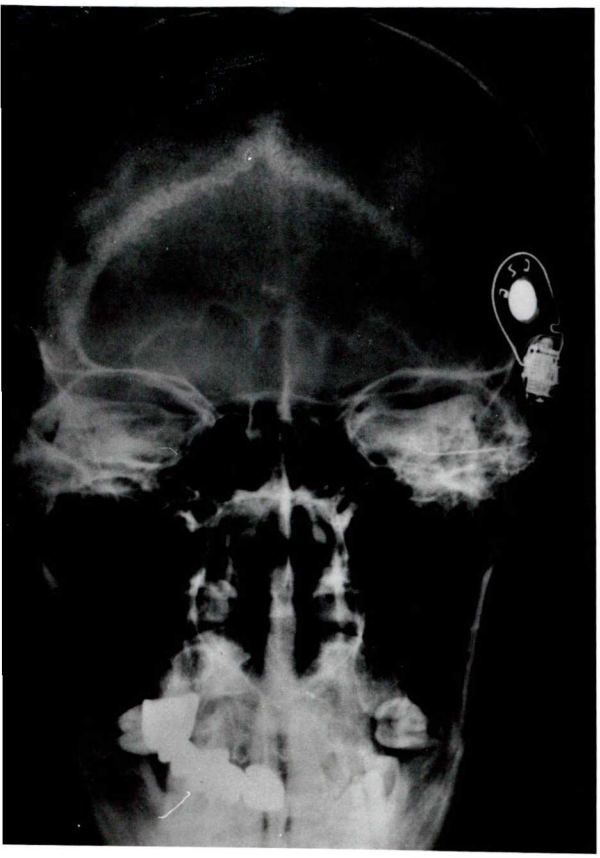


Figure 4b. High resolution CT scans. Patency of the basal turn of the cochlea is seen on both sides.



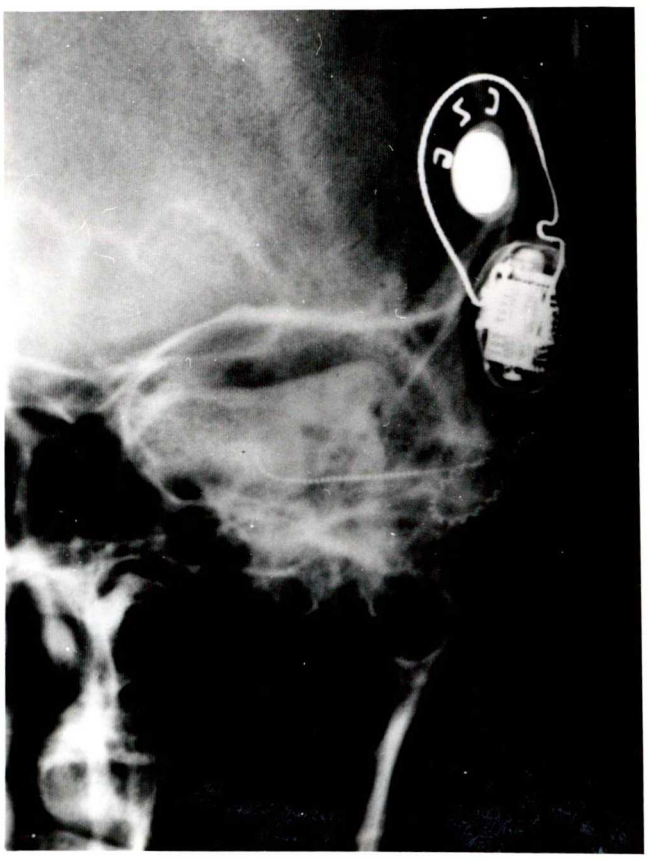


Figure 5. Inappropriate electrode positioning. Some electrodes locate outside the cochlea. (The patient experienced eye and facial twitching upon switch on the device)

Indication for use

General clinical criteria

Cochlear implantation is intended to restore a level of auditory sensation via electrical stimulation of the auditory nerve patients who have postlingual, profound sensorineural deafness and who can not significantly benefit from appropriate amplification of sound by means of a hearing aid.

Specific audiological criteria

From audiological point of view, patients with either poor residual hearing levels with hearing aids from 70-90 dB in the frequencies 1000-1500 Hz (these patients discriminate rhythmic pattern of speech only) or those with no residual hearing without hearing aids (these

patients are totally deaf) are candidates for cochlear implantation. However, a relative indication for patients with hearing level ranging from 100 to 110 dB at frequency range: 1000 to 2000 Hz is currently a subject of consideration. These patients can discriminate prosodic factors like rhythm, intonation, and voicing with hearing aids (Löhle, 1994).

The benefit expected: Implant patients are able to detect medium to loud environmental sounds and conversational speech at comfortable listening levels. The device provides improvement in speech recognition with lip reading; aids in the acquisition and improvement of speech reading skills. The device can provide limited improvement in speech recognition without lip reading and limited improvement in the recognition of environmental sounds.

Adverse effects: Implant patients incur the normal risks of surgery and general anaesthesia. The ear surgery may result in infection or bleeding, numbness or stiffness about the ear, injury to or stimulation of the facial nerve, taste disturbances, dizziness, tinnitus, neck pain, and inner ear fluid leak, which may cause meningitis. Implantation of the receiver/stimulator unit may result in a palpable lump behind the ear. Failure of the implanted device could require removal or replacement. The long-term effects of insertion trauma and chronic electrical stimulation are still the questions of debate. These effects may preclude replacement of the electrode array or may lead to eventual deterioration of cochlear response. Nevertheless, histological studies in deceased cochlear implant users have shown that formation of a fibrous tube surrounding the electrode carrier is often the sole reaction observed. This was the sole event with inert electrode carriers, provided that the intracochlear structure had not been grossly damaged, and the infections had been appropriately prevented (Clark et al., 1988, Linthicum et al., 1991).

It is not possible to say that each adult suffering from postlingual sensorineural deafness benefit from the use of cochlear implants. We have to be completely sure that (1) the patients can not be successfully treated with hearing aid even of excellent quality, (2) the hearing nerve is functionally intact, and (3) the patient is psychically stable, and that the level of intelligence meets the requirements of the postoperative hearing education program. In Hungary, the first clinical criteria for cochlear implantation was provided by Ribari's group in 1989 (Speer et al., 1989).

Recommended preoperative investigations

Rutin medical check-up
Rutin oto-rhino-laryngological check-up
Ophthalmological check-up
Vestibular check-up
Detailed audiological control
- threshold audiometry
- impedance audiometry
- BERA
- otoneurological control

Radiological control: X-ray control, CT, MRI
in some patients, explorative tympanotomy
estimation of the level of intelligence
Studies on psycho-linguistic functions

Korpassy et al. (1992) have published that the 'Promontorium test' is of major importance to estimate the functional capacity of hearing neural system.

The present study does not intend to provide a detailed description of various surgical techniques of implant surgery. Nevertheless, some aspects of surgery are tangentially discussed in relation to the importance of pre- and postoperative investigations.

As multiple-channel stimulation has been shown to provide more information for the understanding of speech than conventional single channel stimulation there is a need to optimise the placement of the electrodes to provide maximal channel capacity. It has been shown that this can be achieved in part by placing the electrode array closer to the spiral ganglion cells which in the human lie within the central axis of the cochlear spiral of modiolus. Not only could this improve channel capacity, but it would reduce the threshold of stimulation and hence the power required to drive receiver/stimulator unit. The aim of the surgery is therefore at least in part to optimize the electrode array position within the scala tympani. In our department the 'soft surgery' technique elaborated by Lehnhardt & Laszig (1993) is applied. The results suggest that the optimum placement of a scala tympani electrode array is close to the modiolus (The Human Communication Research Centre : Fifth annual report 1992). It is accepted that the cochlear implant surgery is not difficult technically (House, 1982, Clark, et al., 1984). The underlying disease (causing deafness), however may influence the anatomical conditions of the cochlea and the round window, that may cause difficulties during and after surgery (Ito, 1993). The important considerations in cochlear implant surgery are therefore to evaluate the condition of the round window and the patency of the cochlea and to confirm the insertion of the electrode array in the cochlea. Besides conventional X-ray control (Rosenberg et al., 1987), CT and MRI (Harnsberger et al. 1987) are the recommended methods to be used for preoperative radiological evaluation. The patency of the cochlea is readily shown by these methods, although it is difficult to ascertain the condition of the round window, particularly when it is normal and membranous or obstructed by granulations or obliterated by ossification (Ito, 1993). Since it is difficult to visualize the round window by CT or MRI, an explorative tympanotomy under local anaesthesia is recommended to inspect the round window directly with a microscope (Ito, 1993).

Intracochlear insertion depth: Radiological evaluation

Recent advances in multiple-channel intracochlear implantation have generated interest in correlating the individual stimulating electrodes to frequency perception, for example, to develop speech processing schemes that map speech frequencies to the frequency appropriate "place" in the cochlea. For this purpose, it is necessary to precisely document the insertion depth of the electrode array and the location of the individual electrodes. Post-implantation radiological examination is an important tool in this regard. Previously, postoperative radiological examination was only performed in cases of unexpected poor device performance (The Human Communication Research Centre : Fifth annual report 1992).

Animal studies have shown that analysis of the distribution of 2-deoxyglucose in the brainstem might serve as an acceptable method to evaluation the position of the electrodes in the inner ear. The 2-deoxyglucose essentially maps the regions of the brain activated in response to stimulation from the bionic ear (Brown et al., 1990).

Special reports on cochlear implantation
(The author's clinical priorities. Case reports on cochlear implantation in special population)

Case 1

Intracochlear implantation of the deaf/blind

Summary

Cochlear implantation in normally sighted individuals is generally regarded as a medical strategy to serve as a supplement to lipreading skills. Therefore, cochlear implantation of the deaf and blind patient was initially considered an intervention of doubtful benefit producing a limited number of implantations round the world. Nevertheless, when the onset of blindness precedes that of deafness, the adaptive plasticity of the cortex, together with the act of the particular patient's motivation may recruit the deaf/blind patient into a unique group of excellent implant performers (Ramsden et al., 1994). This case report describes that multichannel intracochlear implantation has the potential for playing a major role in rehabilitation of certain deaf/blind individuals and that such patients may be amongst the most worthwhile to consider for cochlear implantation.

Case report

A 39 year old blind man was admitted to our department because of profound deafness. He had suffered from bilateral otorrhoea since his childhood following radical mastoidectomy. He lost hearing on the left side due to labyrinth fistula resulting from cholesteatoma recidivans. Hearing loss on the other side ensued owing to end-organ lesion as a consequence of a relatively symptom-poor labyrinthitis in his age of 34.

At the time of admission, pus and debris were found in both mastoid cavities revealing a lack of effect of the preceding antibiotic therapy and repeated aural toilet. The patient had skin lesions, a history of recurrent oral ulceration with respiratory and skin allergy. These abnormalities together with eye lesions resulting in blindness in his early childhood and the presence of the HLA-DR5 alloantigen, the patient was considered to suffer from Behcet's disease because of the presence of 4 of 5 diagnostic criteria of the disease according to suggestion of the International Study Group of Behcet's disease (1990). The laboratory findings showed normal blood glucose, electrolytes, haematology, and liver function tests. No cochlear microphony was observed, and there was no response to caloric stimulation with hot and cold air in either ear. An audiogram demonstrated a profound hearing loss (Figure 6, line A). The promontory test however, exhibited positive results i.e. electrical excitability of the acoustic nerve was seen in both sides, subjectively confirmed by bilateral auditory sensation upon stimulation. Ultra high definition CT scanning showed normal definition and a normal basal turn of the cochlea on both sides. Previously, he was rejected for cochlear implantation because of the otorrhoea possibly deriving from an incomplete removal of the secernating mucosa on occasions of mastoidectomies. In other respects he was considered a good candidate for a multichannel device.

Surgery

To overcome the problem evoked by the lack of a dry protected place to put the electrodes and the device to be implanted, he was submitted to two-stage surgery according to Gray and Irving (1995). In brief, a post-auricular incision was made to approach the mastoid, the pinna was dissected and the external auditory canal was transected at the junction of bony and cartilaginous meatus. Fat was taken from the anterior abdomen. This was followed by a revision mastoidectomy. All residual middle ear mucosa was removed together with the infected mastoid air cells. The mucosa of the Eustachian tube was also removed and its lateral wall was drilled away. The lumen was obstructed with muscle and bone pate. The bony cavity was then polished and a silicone rubber segment was positioned over the promontory and round window. The cavity was obliterated with free abdominal fat. This was then followed by blind sac closure of the external auditory canal according to description by Gray and Irving 1995).

Three months later, the ear was re-opened. The intended site of the implant was marked and a wide incision made. The site for the implant body was prepared. The fibrous fat was retracted forwards and disturbed to achieve access. The previously placed silicone disc was elevated and removed from over the round window and promontory. This was followed by cochleostomy and implantation of a Nucleus 22 ('Cochlear' GmbH Basel, Switzerland) multichannel device. All the 22 electrodes were positioned within the cochlea. The device was fixed near the round window, The wound was closed and the patient was discharged on the third post-operative day. One week after surgery, the correct intracochlear positions of the 22 electrodes were confirmed by means of conventional radiograph in the transorbital view. One month following implantation the device was activated by the audiologist.

Assessment of acoustic discrimination using the cochlear implant

We recorded cognitive responses as determined by the most commonly investigated components of cognitive event-related potentials such as the mismatch negativity (MMN), the N2b and the P300 to study the central auditory processes at the use of the implanted device. The study was performed using a DANTEC CONCERTO EEG-EP equipment (St

Louis, MO) at an experimental setting of the so called 'acoustic oddball paradigm'. The essence of this technique is that the patient is asked to calculate the number of target or deviant acoustic stimuli intervening series of non-target or frequent stimuli. The acoustic oddball paradigm was used under passive condition (the patient read a book for the blind over the investigation period) to verify the MNN and active conditions (the patient was asked to press a button after he had recognized the deviant stimuli) to confirm the other components (N2b and P300) (Regan, 1989; Oviatt & Kileny, 1991; Kraus et al., 1992). The stimulation protocol included a 1000 Hz frequent and 2000 or 500 Hz deviant stimuli.

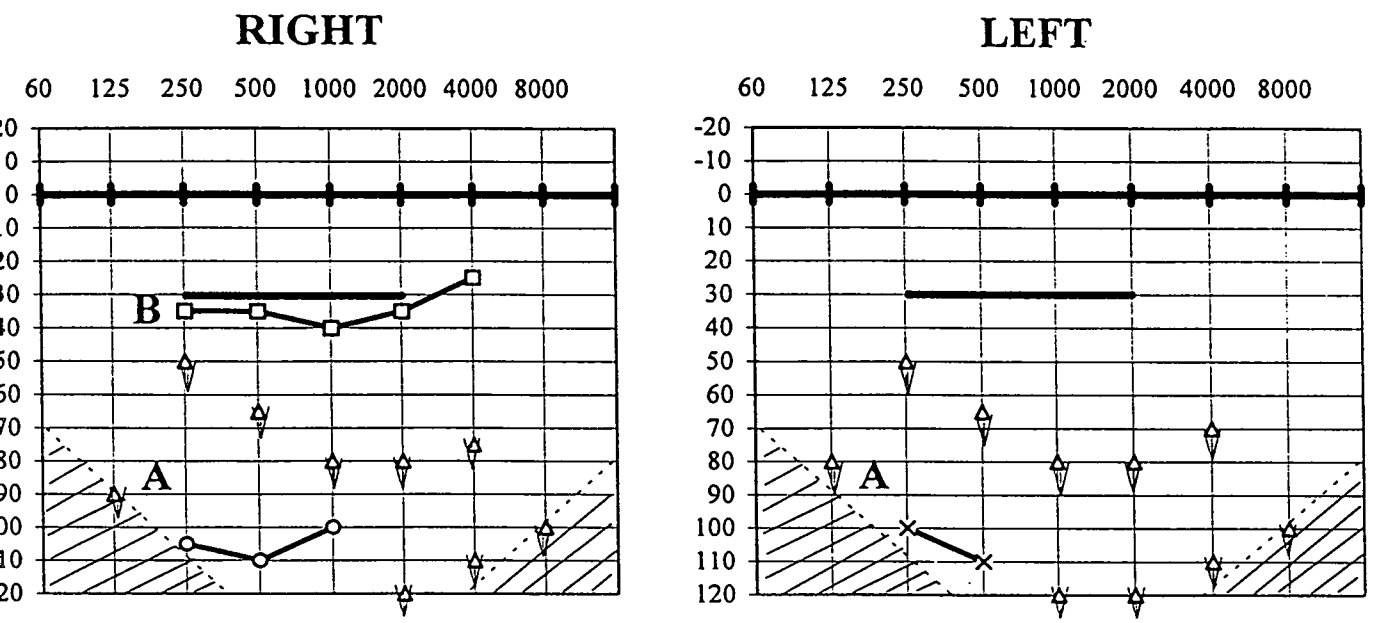


Figure 6. Conventional audiogram. Line A: before implantation; Line B: after implantation. Good performance on the implanted side. Ordinate indicates hearing level in dB; the abscissa indicates frequency in Hz.

Results

Following switch on of the device, the patient acquired an immediate open set speech discrimination ability and continued to improve since then. As objectively indicated by results obtained either from conventional audiology (Figure 6, line B) or the auditory event-related potential study, the patients captured an acoustic discrimination skill comparable to that seen in normally hearing subjects. Nevertheless, the implanted patient revealed a somewhat longer latency period (Figure 7/a) with a smaller amplitude compared to that experienced with the normally hearing individuals (Figure 7/b).

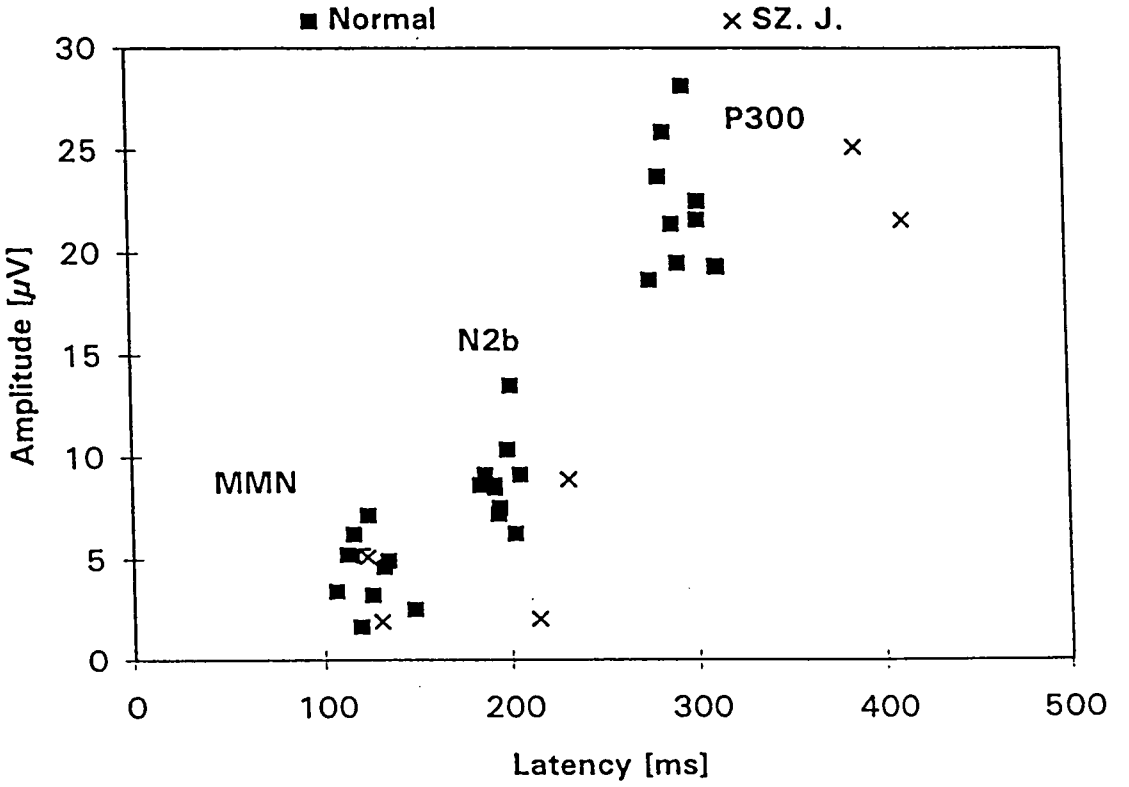


Figure 7 a. Auditory event-related potentials in a deaf/blind patient (initials: SZ.J.) with a Nucleus 22 multichannel intracochlear implant. A comparison with normally hearing subjects. x values indicate individual data of two repetitive determinations with the implanted patient; the black squares denote two repetitive determinations with five normally hearing individuals. An amplitude latency relationship for mismatch negativity (MNN) (passive component and active components (N2b and P300). a: 1000 Hz standard and 2000 Hz deviant stimuli.

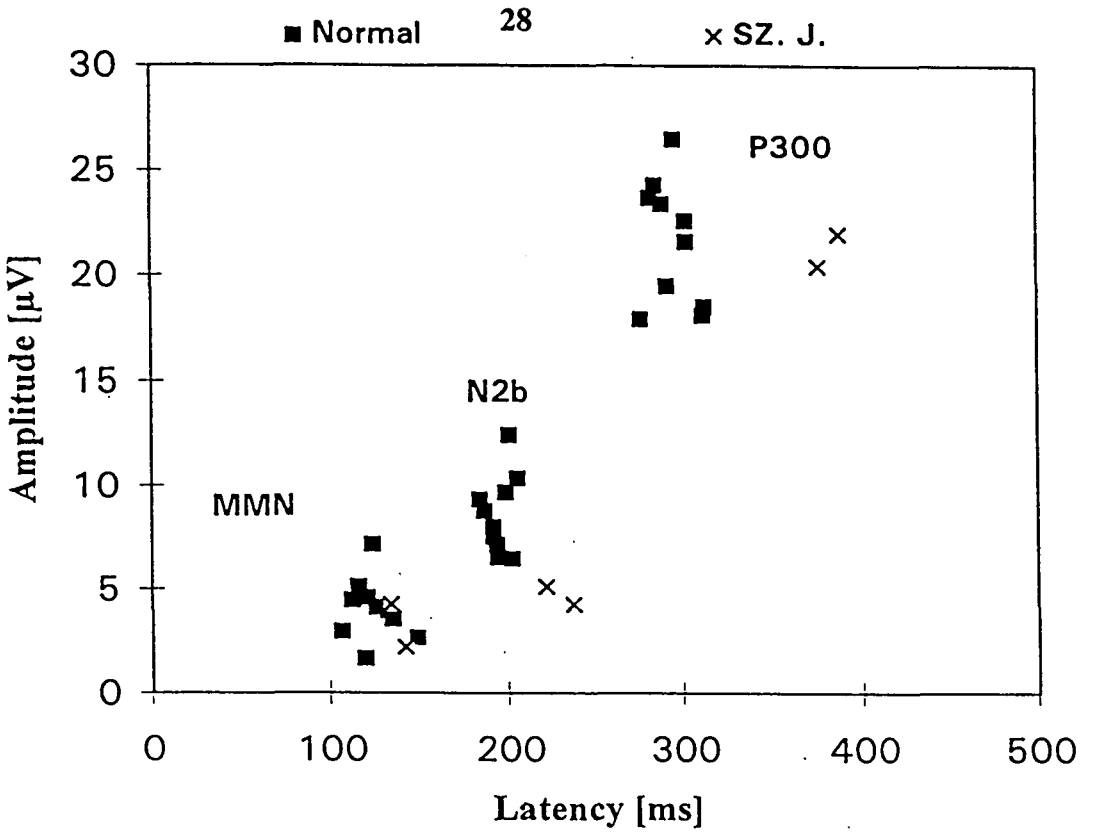


Figure 7b: 1000 Hz standard and 500 Hz deviant stimuli.

Discussion

The results presented show that the deaf blind patient can greatly benefit from intracochlear implantation of a multichannel device. As indicated subjectively by an immediate capture of open set speech discrimination ability subsequent to switch on the device (one month after surgery) and an excellent acoustic discrimination performance at the same time, the patient has reseized his auditory communication skills with the outside world practically immediately following switch on at a baseline level.

The first deaf and blind adult patient receiving Nucleus 22 multichannel intracochlear implant

in Hungary underwent surgery in our department. He is the second deaf/blind Hungarian individual having intracochlear implant and the first one with Nucleus 22 (The 1st deaf/blind Hungarian patient receiving an intracochlear implant is a child implanted with a Med-El device (Ribari et al., 1997). This multihandicapped patient was of particular interest for several reasons. Primarily, because of his multi-sensory organ failure, secondly, since the profoundly deafened patient had been suffering from bilateral discharging ear for decades subsequent to radical mastoidectomy that took place in his childhood. Thus, the patient, otherwise suitable for cochlear implantation was rendered inappropriate for surgery because of the lack of a sterile, dry protected place to put the electrodes. Thirdly, since he had a history of several gastrointestinal and respiratory disorders including gastric ulceration combined with gastrointestinal bleeding and respiratory allergy, we suspected a system disease behind his virtually divers problems.

Cochlear implantation of the deaf/blind individuals previously was thought a risky taking this intervention into account to improving lipreading skills in normally sighted patients. This, at least in part explains why only a relatively small number of deaf/blind have been implanted round the world. Furthermore, the risk of cochlear implantation is further amplified by the additional complication of chronic bilateral discharging ear excluding implantation without preceding preconditioning surgery. Indeed, our patient had also been refused to be considered a candidate for intracochlear implantation because of the discharging ears at another implantation centre. We therefore decided to recondition the ear for cochlear implantation using the internationally accepted preconditioning surgery proposed by Gray & Irving (1995). Subsequently, we found no difficulties in the implantation procedure.

It is difficult to estimate the reason as to whether why our patient disclosed such an excellent auditory performance immediately subsequent to device activation. According to the experiences of Ramsden et al. (1994) the deaf/blind have been amongst the very best performers. For an explanation, it is suggestive that humans individuals similar to several animal species, who had been blind for years prior to the onset of deafness are able to better utilize auditory information than one might expect, because adaptive plasticity of the cortex. The situation is fairly different in patients who lose sight and hearing simultaneously or in those with preceding deafness. Whatever the precise explanation is, we think that deaf/blind patients with preceding blindness can much better benefit from multichannel intracochlear implantation than thought previously.

Case 2

Intracochlear implantation in an epileptic patients with generalized seizures.

Summary

A case is reported in which a Nucleus 22 channel intracochlear implant was applied to a Hungarian deaf women (age 37 yr) with a 34 yr history of GM epilepsy maintained on carbamazepine-diazepam combination therapy who had not benefit from conventional hearing aids. Preoperative electrical stimulation of the acoustic nerve, however, exhibited a good nerve function with no evidence for abnormal waveforms in the electroencephalogram (EEG). Successful intracochlear insertion of the 22 electrode resulted in a 40 dB hearing improvement at frequencies 250-2000 HZ in the implanted ear with no signs of pathologic wave activity at neither the previously recognized epileptic focus (fronto-precentralis region) nor other regions of the brain at use of the implant. We conclude that intracochlear implantation 'per se' does not serve as a hazardous intervention in patients with fronto-precentralis epileptic foci.

Classically, the use of cochlear implants can be regarded as an intervention of particular risk in patients with epilepsy. The electrical field that stimulates neural elements around the implanted electrodes often results in non-acoustic nerve activity reflected in eye twitching, painful sensations facial twitching. Muller-Deile et al., 1994). At certain conditions, however, seizure discharges can also be produced, moreover, these discharges may become self-sustaining beyond the original stimulus (Laidlaw & Richens, 1988). Evolution of this mechanism is certainly facilitated by a preceding development of an epileptic focus. Thus, it is not surprising that a very limited number of patients have received cochlear implants. According to our best knowledge, no detailed clinical report has been presented on intracochlear implantation of a multichannel device in clinical patients with generalized epilepsy. This case report describes that appropriate positioning of the intracochlear electrodes with an adequate pharmacological antiepileptic control make cochlear implantation safe under condition of generalized epilepsy.

Case report

A 37 year old epileptic women was admitted to our department because of profound deafness. She had suffered from generalized epilepsy since her age of 3 due to unknown reason. Her family belonged to a very low socioeconomic class. She had been using several conventional hearing aids with an initial minimum and later no benefit. At the time of admission, she was unable for verbal communication without lipreading. Epilepsy was controlled with a carbamazepine-chlordiazepoxid combination therapy. She was free of seizures in the last 10 years.

She was evaluated and considered a suitable candidate for cochlear implantation. High definition CT scanning of the temporal bones at the time of evaluation did not reveal any abnormalities and indicated patent cochleas on both sides. An audiogram demonstrated a profound hearing loss on both sides (Figure 8). The promontory test exhibited positive results i.e. electrical excitability of the acoustic nerve was seen on both sides, subjectively confirmed by bilateral auditory sensation upon stimulation. Accomplishment of the promontory test failed to provoke epileptic seizures. The patient preferred the right side for implantation because she felt that it would be easier for her to use the telephone.

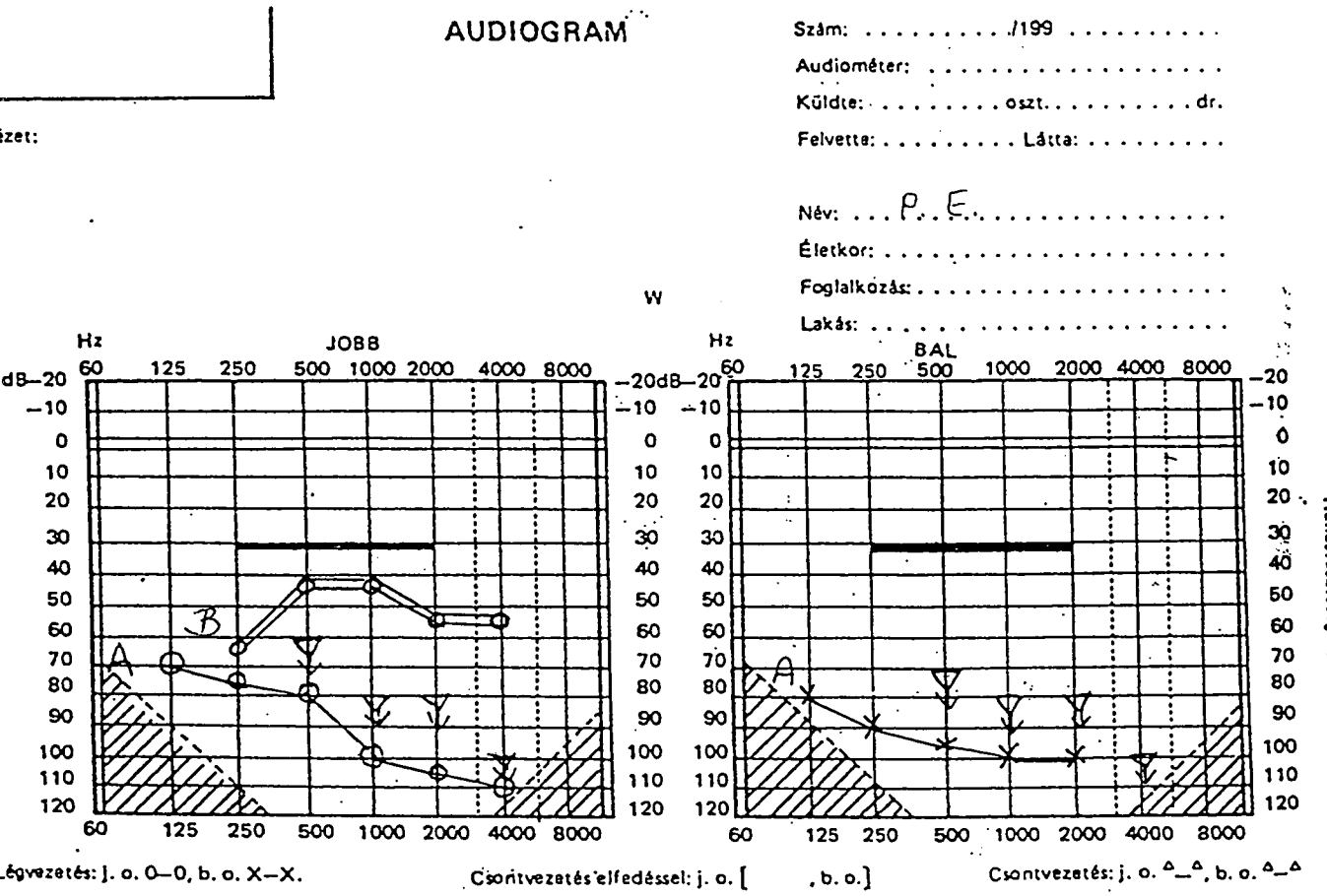


Figure 8. Conventional audiogram. Line A: before implantation; Line B: after implantation. Good performance on the implanted side.

Prior to implantation the patient mostly relied on lipreading since she could no longer benefit from conventional hearing aids. Therefore, she was well motivated and psychological evaluation revealed no adverse features. We considered her gradual hearing loss a good prognostic parameter. A meticulous neurological evaluation was carried out prior to surgery with special regards to potential risk of seizure provocation. Routine scalp-recorded electroencephalogram (EEG) provided no evidence for electrographic seizure activity i.e. abnormal, repetitive rhythmic activity having an abrupt onset and termination (Aminoff, 1992). Deprivation of sleep on the night prior to recording, however, exhibited focal activity over the right precentralis area (Figure 9, left panel) in the absence of antiepileptic drug therapy. The combination therapy was re-applied after completion of the sleep deprivation EEG study.

We inserted all 22 electrodes of a ^Nucleus 22^ device into the scala tympany via a basal turn cochleostomy. Post-operative plain X-rays showed normal position of the implant (Figure 10).

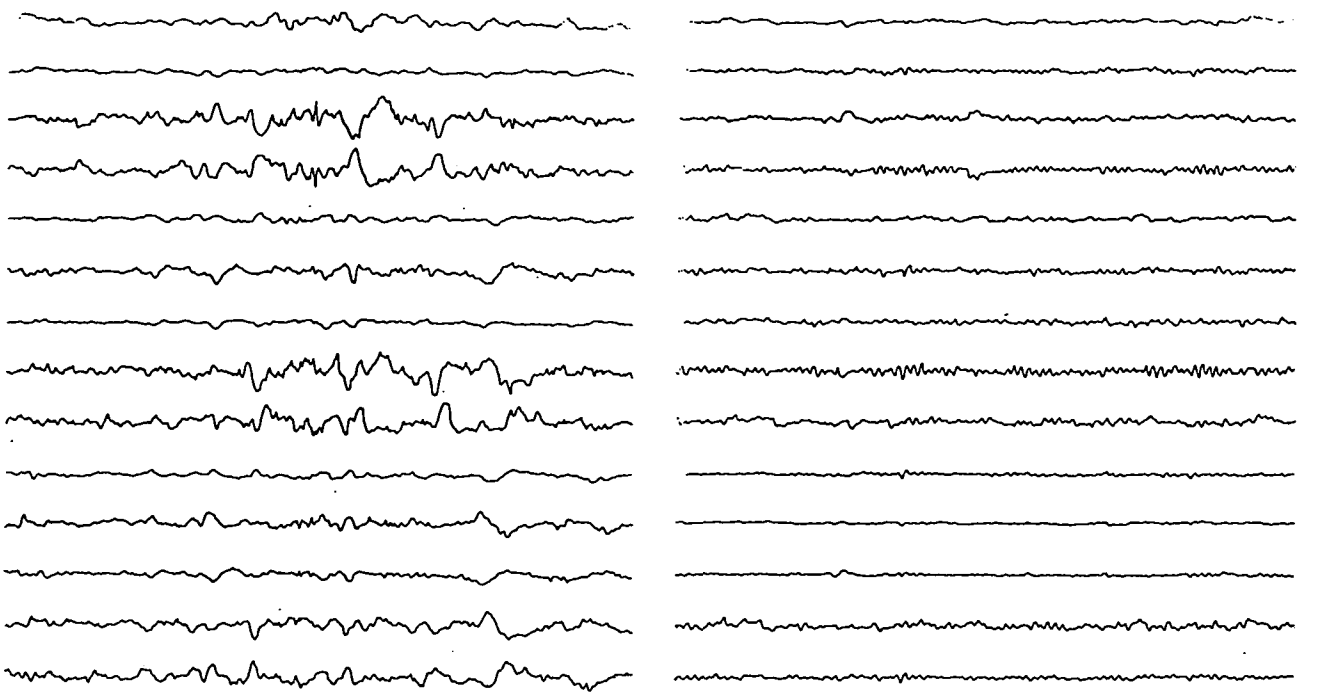


Figure 9. Effect of sleep deprivation before implantation (left panel) and intracochlear implantation (right panel;the patient was listening to music) on electrical activity of the brain. Investigations in a patient with a 34 yr history of GM epilepsy. EEG electrodes are positioned according to the international 10:20 system.

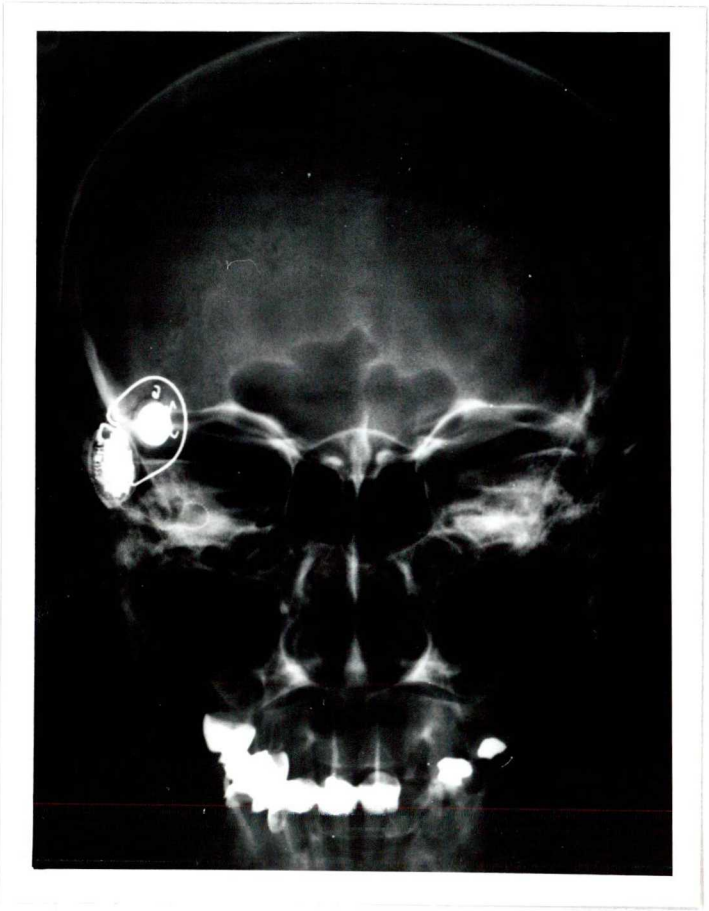


Figure 10a. Intracochlear electrode positioning. Each of the 22 electrodes is inserted into basal turn of the cochlea. Trans-orbital Stenvers view.

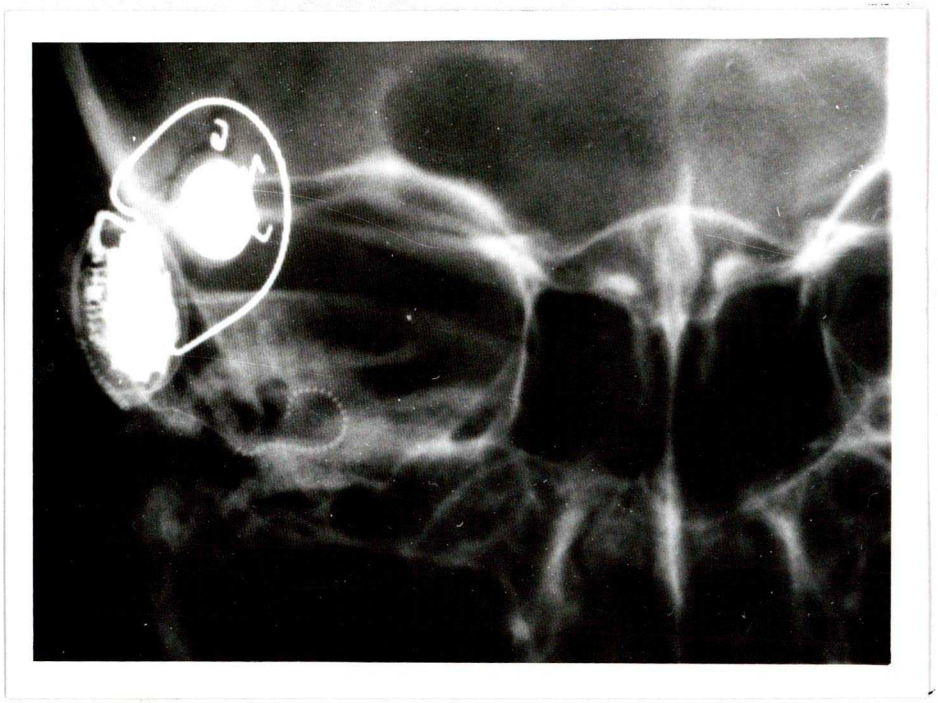


Figure 10b. Intracochlear electrode positioning. Each of the 22 electrodes is inserted into basal turn of the cochlea. Trans-orbital Stenvers view. Enlargement of the relevant area.

On the third post-operative day, a routine electrode checking was carried out. No evidence for extra-auditory nerve stimulation was seen at either common ground or bipolar+1 stimulation mode. Postoperative audiology revealed a significant improvement of hearing in the implanted ear (Figure 8). At one month the patient was able to identify 20/20 of everyday sounds and connected discourse tracking exhibited a high level of open set speech discrimination. The auditory event-related potential study confirmed that the patients captured an acoustic discrimination skill comparable to that seen in normally hearing individuals. The patient was free of epileptic episodes at use of the implant, similarly, the EEG did not disclose any epileptic pattern when the patient was listening to music (Figure 9, right panel)

Discussion

According to or best knowledge, this case report is the first to describe that an epileptic patient suffering from generalized seizures benefits from intracochlear implantation. As indicated by a significant improvement of the patient's hearing subjectively and shown quantitatively by results from conventional audiology and the auditory event-related potential study, the use of the cochlear implant improved hearing to a degree similar to that seen with normally hearing individuals with no evidence for either clinical or electrographic seizure provoking effect. Alternatively, it is also shown that neither epilepsy nor antiepileptic pharmacotherapy produced a negative influence on cochlear implant function.

Epileptic seizures can be induced in any normal vertebrate brain with a variety of electrical (or chemical) stimuli (Laidlaw & Richens, 1988). At certain current strengths and stimulus frequencies, seizure discharges are produced and may become self-sustaining beyond the original stimulus. At lower stimulus parameters, only brief afterdischarges occur. Nevertheless, if afterdischarge-provoking stimuli are repeated at regular intervals, the afterdischarge spread and duration throughout the brain may increase until generalized seizures arise to the same range of stimuli which was originally subthreshold (Dichter & Ayala, 1987). Eventually, spontaneous seizures may occur even in the absence of electrical stimulation. However, the question as to whether this mechanism termed *kindling* contributes to a considerable degree to human epilepsy is still a question of debate. Whatever the mechanism is, long lasting electrical stimulation of the epileptic brain (as occurs with cochlear implantation) necessitates cautiousness, since these patients may be more vulnerable to electrical stimulation-induced seizures than normal individuals.

When an epileptic focus with isolated discharges receives further excitatory impulses for example deriving from external electrical stimulation, it may undergo a transition from the isolated discharges to a seizure (Dichter, 1994). Speculatively, contiguous areas and synaptically connected distant areas exposed to electrical stimulation may facilitate such a development of generalized epileptic seizures. Alternatively, when there is no direct connection between group of the externally stimulated group neurons (spiral ggl. cells) and the existing epileptic focus (fronto-precentralis area), any seizure provoking effect of cochlear

field stimulation is uncertain. Thus, the fronto-precentralis localization of the potentially epileptogenic focus in this case almost excludes the possibility of a superposing epilepsy of acoustic origin. In the present case, however, the intracochlear localization of all the 22 electrodes restrains impulses by the implanted stimulator to the acoustic nerve. Indeed, no additional peripheral nerve excitation was seen at either electrode programming protocol. These results seem to be in accordance with those presented as tangential publications by Gibbin et al. (1994) and Clark (1997) who did not found any complication with cochlear implantation of epileptic children at appropriate antiepileptic pharmacotherapy.

In summary, the results presented in this case report suggest that GM epilepsy 'per se' is not a clinical contraindication for intracochlear implantation.

Concluding remarks

The author hypothesized that cochlear implantation can be considered a particular type of electrical field stimulation according to computed signals. The selectivity of stimulation can be assured by appropriate electrode positioning and speech programming. It is the only instance in which a lost sense can be restored to a significant degree of function. In an overwhelming majority of cases, children and adults who have lost their hearing due to trauma, infection, treatment with toxic drugs or other causes can be helped by these implants. Even more exciting is the fact that these devices can enable children who were born deaf or who lost hearing prior to acquisition of speech to learn to speak, so they can attend to schools instead of schools for the deaf and they do not have to depend solely on sign language for communication. The ^Author's own priorities^ section gets an insight into perspectives for special multihandicapped deaf populations such as the deaf/blind and epileptics.

Conclusions from the experimental part seem to support the concept that the endothelial layer is a transducer of important vascular signals to which the vascular bed must respond in order to ensure adequate tissue perfusion according to moment-to-moment requirements. Moreover, this vascular signal may be a target for neural inputs in the cochlea. Our results also imply a possible beneficial effect of pharmacologic manoeuvres that utilize the cyclic GMP pathway to support the clinical use of cochlear electrical stimulation.

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Köszönetnyilvánítás

Köszönetemet fejezem fejezem ki Czigner professzornak, főnökömnek, aki pályámon elindított, tanított és segített abban, hogy munkám elkészüljön.

Hálásan köszönöm munkahelyi szakmai vezetőm, Dr Kiss József Géza tudományos irányítását, aki a szakmán belül bevezetett az audiologia és az otoneurologia világába.

Köszönöm Jancsó professzornak munkám tudományos irányítását, büszke vagyok arra, hogy az általa képviselt tudományból néhány sugár rám vetült.

Hálával tartozom Jóri József tanár Úrnak, akitől a cochleáris implantáció kivitelezésében értékes segítséget kaptam.

Köszönöm a segítséget valamennyi szerzőtársamnak, különösen Dr Ferdinandy Péternek aki nagyban segített a kísérletek helyes irányba terelésében.

Külön köszönet illeti Bencsik Sándorné állatgondozót és Hatvani Ilona asszisztensnőt, akik a kísérleti munkában értékes technikai segítséget nyújtottak.

Hálával tartozom a SZOTE Fül-Orr-Gégeklinika összes dolgozójának, kollégáimnak; az ő mindennapos kollegiális segítsége néklül ez a munka nem készülhetett volna el.

The thesis is based on publications as follows

Clinical chapter

1, Szilvássy J, Czigner J, Jori J, Toth F, Szilvássy Z, de Mora Mieszkowski J, Kiss JG. Cochlear implantation of a Hungarian deaf and blind patient with discharging ears suffering from Behcet's disease. J. Laryngol & Otol. 112, 169-171, 1998.

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The Journal of Laryngology and Otology
February 1998. Vol. 112, pp. 169–171

Cochlear implantation of a Hungarian deaf and blind patient with discharging ears suffering from Behçet's disease

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Abstract

A case is reported in which a Nucleus 22 channel intracochlear device was implanted a deaf/blind Hungarian adult with discharging ears suffering from Behçet's disease. Preconditioning surgery was employed three months prior to the implantation procedure to ensure a sterile, dry protected environment for the electrodes. One month after implantation, the patient exhibited excellent auditory discrimination capability at the time of the first switch on. We suggest that some deaf/blind individuals may serve as very good candidates for intracochlear implantation.

Key words: Cochlear implant; Behçet's syndrome

Introduction

Cochlear implantation in normally-sighted individuals is generally regarded as a medical strategy which serves to supplement lipreading skills. Cochlear implantation of the deaf and blind patient was therefore initially considered an intervention of doubtful benefit and there have only been a limited number of implantations round the world. Nevertheless, when the onset of blindness precedes that of deafness, the adaptive plasticity of the cortex, together with the motivation of the patient may recruit the deaf/blind patient into a unique group of excellent implant performers (Ramsden *et al.*, 1994). This case report suggests that multichannel intracochlear implantation has the potential for playing a major role in the rehabilitation of certain deaf/blind individuals and that such patients may be amongst the most worthwhile to consider for cochlear implantation.

Case report

A 39-year-old blind man was admitted to our department because of profound deafness. He had suffered from bilateral otorrhoea since childhood following radical mastoidectomy. He lost hearing on the left side due to a labyrinthine fistula resulting from cholesteatoma. Hearing loss on the other side ensued as a result of an end-organ lesion as a consequence of labyrinthitis when he was 34 years old.

At the time of admission, pus and debris were found in both mastoid cavities despite antibiotic therapy and repeated aural toilet. The patient had skin lesions and a history of recurrent oral ulceration with respiratory and skin allergy. These abnormalities together with eye lesions resulting in blindness in his early childhood and the presence of the HLA-DR5 alloantigen, suggested the patient was suffering from Behçet's disease because of the presence of four of five diagnostic criteria of the disease according to suggestion of the International Study Group of Behçet's disease (1990). The laboratory findings showed normal blood glucose, electrolytes, haematology, and liver function tests. An audiogram demonstrated a profound hearing loss (Figure 1). No cochlear microphonic was observed, and there was no response to caloric stimulation with hot and cold air in either ear. The promontory test however, exhibited positive results ie. electrical excitability of the acoustic nerve was seen in both sides, subjectively confirmed by bilateral auditory sensation upon stimulation. Ultra high definition computed tomography (CT) scanning showed normal definition and a normal basal turn of the cochlea on both sides. Previously, he was rejected for cochlear implantation because of the otorrhoea possibly deriving from an incomplete removal of the secreting mucosa at previous mastoidectomy. In other respects he was considered a good candidate for a multichannel device.

Surgery

To overcome the problem of infected mastoid cavities he was submitted to two-stage surgery according to the description by Gray and Irving (1995). A post-auricular incision was made to approach the mastoid, the pinna was dissected and the external auditory canal was transected at the junction of bony and cartilaginous meatus. Fat was taken from the anterior abdomen. This was followed by a revision mastoidectomy. All residual middle-ear mucosa was removed together with the infected mastoid air cells. The mucosa of the Eustachian tube was also removed and its lateral wall was drilled away. The lumen was obstructed with muscle and bone paté. The bony cavity was then polished and a piece of silicone rubber sheeting was

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Accepted for publication: 23 December 1997.

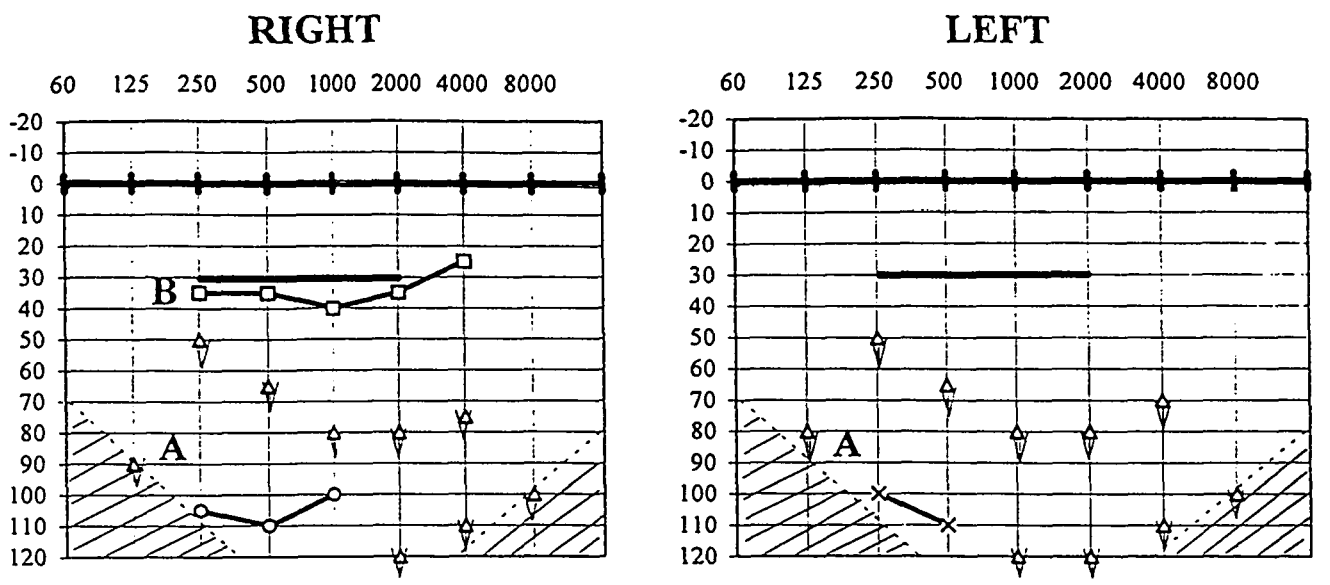


FIG. 1
Conventional audiology.
Curve A: Pre-operative unaided auditory thresholds. Curve B: Cochlear implant thresholds.

positioned over the promontory and round window. The cavity was obliterated with free abdominal fat. This was then followed by blind sac closure of the external auditory canal according to the description by Gray and Irving (1995).

Three months later, the ear was re-opened. The intended site of the implant was marked and a wide incision made. The site for the implant body was prepared. The fibrous fat was retracted forwards for access. The previously placed silicone disc was elevated and removed from over the round window and promontory. This was followed by cochleostomy and implantation of a Nucleus 22 ('Cochlear' GmbH Basel. Switzerland) multichannel device. All the 22 electrodes were positioned within the cochlea. The device was fixed near the round window. The wound was closed and the patient was discharged on the third post-operative day. One week after surgery, the correct intracochlear positions of the 22 electrodes were confirmed on a conventional transorbital radiograph. One month following implantation the device was activated by the audiologist.

Assessment of acoustic discrimination using the cochlear implant

We recorded cognitive responses as determined by the most commonly investigated components of cognitive event-related potentials such as the mismatch negativity (MMN), the N2b and the P300 to study the central auditory processes at the use of the implanted device. The study was performed using Dantec Concerto EEG-EP equipment (St Louis, MO) at an experimental setting of the so-called 'acoustic oddball paradigm'. The essence of this technique is that the patient is asked to calculate the number of target or deviant acoustic stimuli in a series of non-target or frequent stimuli. The acoustic oddball paradigm was used under passive conditions (the patient read a book for the blind over the investigation period) to verify the MNN and active conditions (the patient was asked to press a button after he had recognized the deviant stimuli) to confirm the other components (N2b and P300) (Regan, 1989; Oviatt and Kileny, 1991; Kraus *et al.*, 1992). The stimulation protocol included a 1000 Hz frequent and 2000 or 500 Hz deviant stimuli.

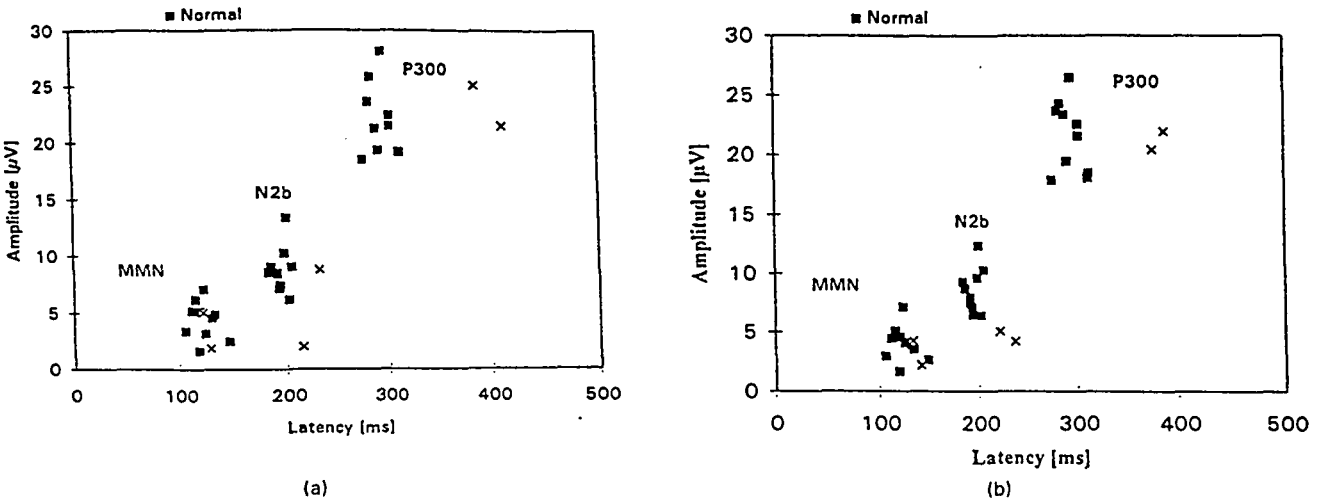
Results

Following switching on of the device, the patient acquired immediate open set speech discrimination ability and has continued to improve since then. As objectively indicated by results obtained from conventional audiology (Figure 1) and the auditory event-related potential study, the patient achieved an acoustic discrimination skill comparable to that seen in normally hearing subjects. Nevertheless, the implanted patient revealed a somewhat longer latency period (Figure 2a) with a smaller amplitude compared to that experienced by normally hearing individuals (Figure 2b).

Discussion

The results presented show that the deaf blind patient can benefit greatly from intracochlear implantation of a multichannel device. As indicated subjectively by immediate achievement of open set speech discrimination ability after switching on of the device (one month after surgery) and an excellent acoustic discrimination performance at the same time, the patient has regained his auditory communication skills with the outside world almost immediately following switch on at a baseline level.

The first deaf and blind adult patient to receive a Nucleus 22 multichannel intracochlear implant in Hungary underwent surgery in our department. He is the second deaf/blind Hungarian individual having an intracochlear implant and the first one with Nucleus 22 (the first deaf/blind Hungarian patient to receive an intracochlear implant is a child implanted with a Med-El device (Ribari *et al.*, 1997). This multihandicapped patient was of particular interest for several reasons. Primarily, because of his multi-sensory organ failure, secondly, since the profoundly deafened patient had been suffering from bilateral discharging ear for decades subsequent to radical mastoidectomy that took place in his childhood. Thus, the patient, otherwise suitable for cochlear implantation was rendered inappropriate for surgery because of the lack of a sterile, dry protected site for the electrodes. Thirdly, since he had a history of several gastrointestinal and respiratory disorders including gastric ulceration combined with gastrointestinal bleeding and respiratory allergy, we suspected a systemic disease.



X values indicate individual data of two repetitive determinations with the implanted patient; the black squares denote two repetitive determinations with five normally hearing individuals. An amplitude latency relationship for mismatch negativity (MNN) (passive component and active components (N2b and P300).
a: 1000 Hz standard and 2000 Hz deviant stimuli; b: 1000 Hz standard and 500 Hz deviant stimuli.

FIG. 2
Auditory event-related potentials in a deaf/blind patient with a Nucleus 22 multichannel intraocochlear implant. A comparison with normally hearing subjects.

Cochlear implantation of deaf/blind individuals was previously thought risky bearing in mind the technique was thought to improve lipreading skills in normally sighted patients. This, technique at least in part, explains why only a relatively small number of deaf/blind have been implanted round the world. Futhermore, the risk of cochlear implantation is further amplified by the additional complication of chronic bilateral discharging ear excluding implantation without preceding pre-conditioning surgery. Indeed, our patient had also been refused intracochlear implantation because of the discharging ears at another implantation centre. We therefore decided to prepare the ear for cochlear implantation using the surgery proposed by Gray and Irving (1995). Subsequently, we found no difficulties in the implantation procedure.

It is difficult to state the reason why our patient showed such an excellent auditory performance immediately after device activation. According to the experiences of Ramsden *et al.* (1994) the deaf/blind have been amongst the very best performers. For an explanation, it is suggested that human individuals, similar to several animal species, who had been blind for years prior to the onset of deafness are better able to utilize auditory information than one might expect, because of adaptive plasticity of the cortex. The situation is different in patients who lose sight and hearing simultaneously or in those with preceding deafness. Whatever the precise explanation is, we think that deaf/blind patients with preceding blindness can derive greater benefit from multichannel intracochlear implantation than previously thought.

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The Journal of Laryngology and Otology
June 1998, Vol. **112**, pp. 000–000

Cochlear implantation in a patient with grand mal epilepsy

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Abstract
A case is reported in which a Nucleus 22 channel intracochlear implant was used to treat a deaf Hungarian woman (aged 37 years) with a 34-year history of grand mal (GM) epilepsy maintained on carbamazepine-diazepam combination therapy who had not benefited from conventional hearing aids. Pre-operative electrical stimulation of the acoustic nerve, however, exhibited a good nerve function with no evidence of abnormal waveforms in the electroencephalogram (EEG). Successful intracochlear insertion of the 22 electrode resulted in a 40 dB hearing improvement at frequencies 250–2000 Hz in the implanted ear with no signs of pathologic wave activity at either the previously recognized epileptic focus (fronto-precentral region) or indeed, in other regions of the brain at use of the implant. We conclude that intracochlear implantation *per se* is not a hazardous intervention in patients with fronto-precentral epileptic foci.

Key words: Cochlear implant; Epilepsy; Electrical stimulation

Introduction

Classically, the use of cochlear implants can be regarded as an intervention of potential risk in patients with epilepsy. The electrical field that stimulates neural elements around the implanted electrodes often results in non-acoustic nerve activity reflected in eye twitching, or painful facial twitching (Muller-Deile *et al.*, 1993). Under certain conditions, however, seizure discharges can also be produced and these discharges may become self-sustaining beyond the original stimulus (Rasmussen, 1983). Evolution of this mechanism is certainly facilitated by the preceding existence of an epileptic focus. Thus, it is not surprising that a very limited number of such patients have received cochlear implants. To our knowledge, no detailed clinical report has been presented on intracochlear implantation of a multichannel device in a patient with generalized epilepsy. This case report describes that appropriate positioning of the intracochlear electrodes with adequate pharmacological antiepileptic control make cochlear implantation safe when generalized epilepsy is present.

Case report

A 37-year-old epileptic woman was admitted to our department because of profound deafness. She had suffered from generalized epilepsy since the age of three for unknown reasons. Her family came from a very low socioeconomic class. She had been using several conventional hearing aids initially with minimal benefit and later without benefit. At the time of admission, she was incapable of verbal communication without lipreading. Epilepsy was controlled with a carbamazepine-chlordiazepoxid combination therapy. She had been free of seizures for the last 10 years.

She was evaluated and considered a suitable candidate for cochlear implantation. High definition computed tomography (CT) scanning of the temporal bones at the time of evaluation did not reveal any abnormalities and indicated patent cochleas on both sides.

An audiogram demonstrated a profound hearing loss on both sides (Figure 1). The promontory test exhibited positive results i.e. electrical excitability of the acoustic nerve was seen on both sides, subjectively confirmed by bilateral auditory sensation upon stimulation. The promontory test failed to provoke epileptic seizures. The patient preferred the right side for implantation because she felt that it would be easier for her to use the telephone.

Prior to implantation the patient mostly relied on lipreading since she could no longer benefit from conventional hearing aids. She was well motivated and psychological evaluation revealed no adverse features. We considered her gradual hearing loss a good prognostic parameter. A meticulous neurological evaluation was carried out prior to surgery with special regard to the potential risk of seizure provocation. Routine scalp-recorded electroencephalogram (EEG) provided no evidence of electrographic seizure activity i.e. abnormal, repetitive rhythmic activity having an abrupt onset and termination (Gibbin *et al.*, 1992). Deprivation of sleep on the night prior to recording, however, revealed focal activity over the right precentralis area (Figure 2a) in the absence of antiepileptic drug therapy. The combination therapy was re-applied after completion of the sleep deprivation EEG study.

We inserted all 22 electrodes of a nucleus 22 device into the scala tympani via a basal turn cochleostomy. Post-operative plain X-rays showed the normal position of the implant.

On the third post-operative day, a routine electrode check was carried out. No evidence of extra-auditory nerve stimulation was seen at either the common ground or bipolar + 1 stimulation mode. Post-operative audiology revealed a significant improvement of hearing in the implanted ear (Figure 1). At one month the patient was

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Accepted for publication: 15 April 1998.

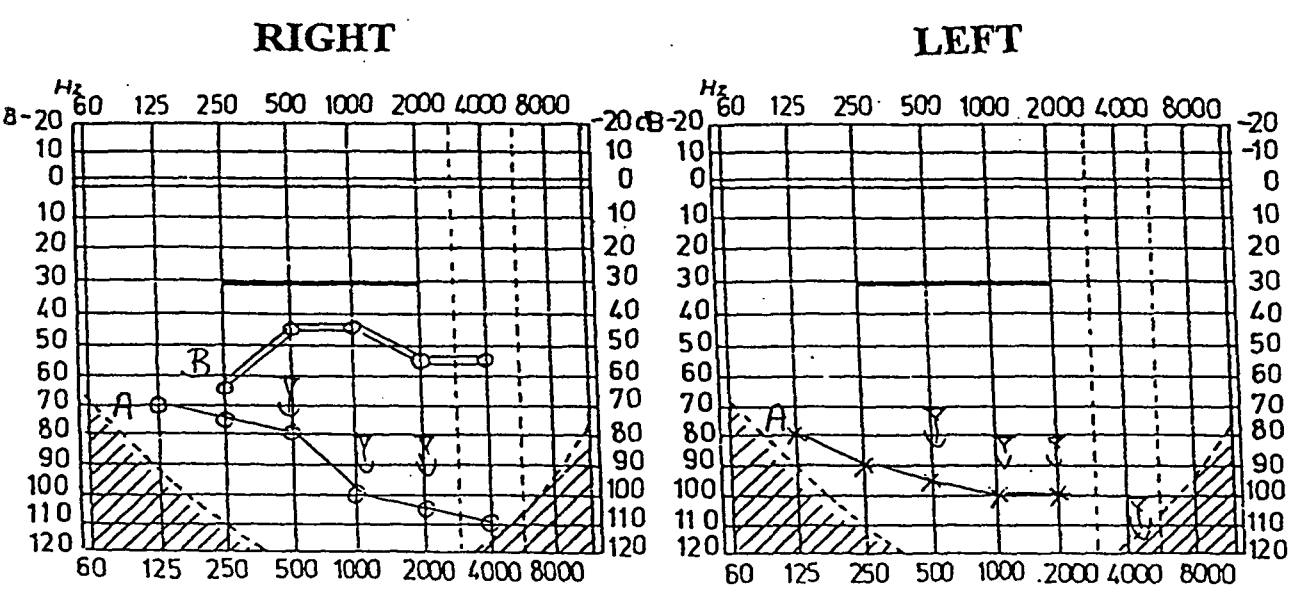

FIG. 1

Conventional audiogram. Line A: before implantation; Line B: after implantation. Good performance on the implanted side.

le to identify 20/20 of everyday sounds and connected course tracking exhibited a high level of open set speech crimination. The auditory event-related potential study cording to Oviatt *et al.* 1991) confirmed that the patient ained an acoustic discrimination skill comparable to that n in normally hearing individuals. The patient was free epileptic episodes when using the implant, and, the G did not disclose any epileptic pattern when the ient was listening to music (Figure 2b).

Discussion

To the best of our knowledge, this case report is the first to describe that an epileptic patient suffering from generalized seizures benefits from intracochlear implantation. As indicated by a significant improvement in the patient's hearing subjectively and as shown quantitatively by results from conventional audiology and the auditory event-related potential study, the use of the cochlear implant improved hearing to a degree similar to that seen

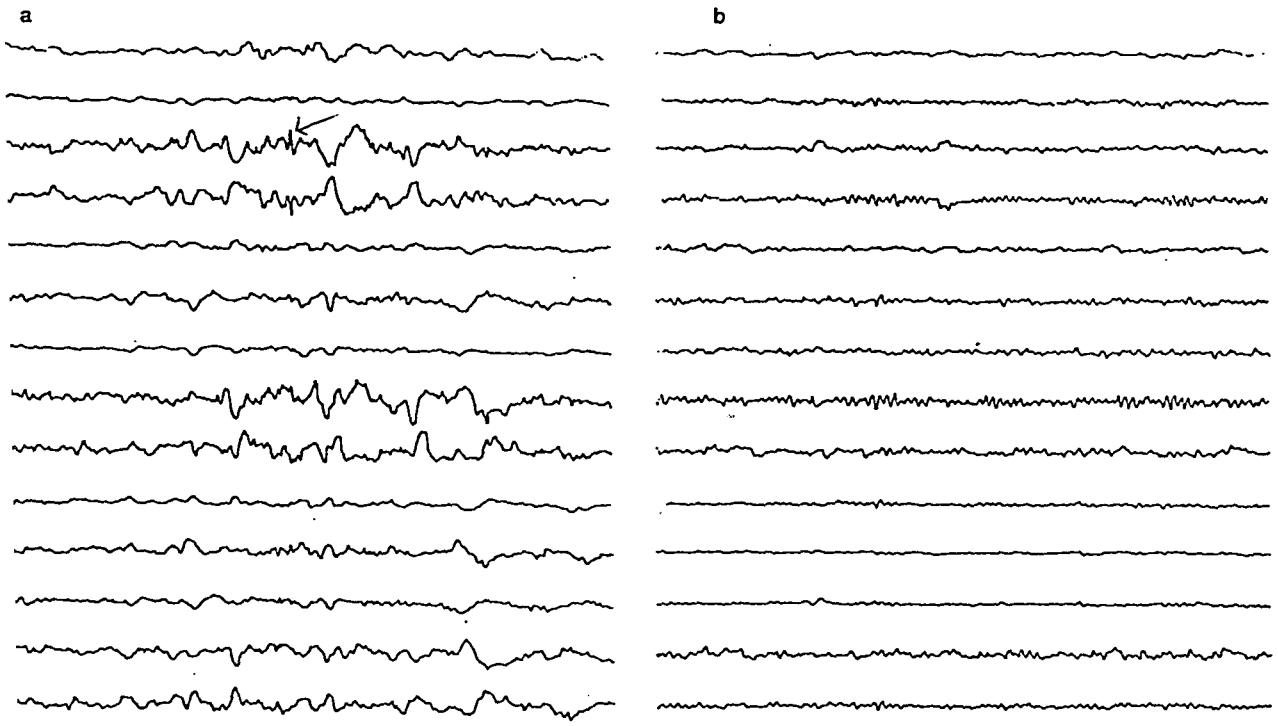

FIG. 2

ct of sleep deprivation before implantation (a) and intracochlear implantation ((b); the patient was listening to music) on trical activity of the brain. Investigations in a patient with a 34-year history of GM epilepsy. EEG electrodes are positioned ccording to the international 10:20 system. The arrows show pathologic wave patterns (spikes) characteristic for epilepsy.

in normally hearing individuals with no evidence of either clinical or electroencephalographic seizure-provoking effect. It is also shows that neither epilepsy nor anti-epileptic pharmacotherapy produced a negative influence on cochlear implant function.

Epileptic seizures can be induced in any normal vertebrate brain with a variety of electrical (or chemical) stimuli. At certain current strengths and stimulus frequencies, seizure discharges are produced and may become self-sustaining beyond the original stimulus. At lower stimulus parameters, only brief after discharges occur. Nevertheless, if after discharge-provoking stimuli are repeated at regular intervals, the afterdischarge spread and duration throughout the brain may increase until generalized seizures arise to the same range of stimuli which was originally subthreshold (Dichter and Ayala, 1987). Eventually, spontaneous seizures may occur even in the absence of electrical stimulation. However, the question as to whether this mechanism termed *kindling* contributes to a considerable degree to human epilepsy is still a question of debate. Whatever the mechanism, long-lasting electrical stimulation of the epileptic brain (as occurs with cochlear implantation) necessitates caution, since these patients may be more vulnerable to electrical stimulation-induced seizures than normal individuals.

When an epileptic focus with isolated discharges receives further excitatory impulses for example, deriving from external electrical stimulation, it may undergo a transition from the isolated discharges to a seizure (Dichter, 1994). Speculatively, contiguous areas and synaptically connected distant areas exposed to electrical stimulation may facilitate such a development of generalized epileptic seizures. Alternatively, when there is no direct connection between groups of the externally stimulated group neurons (spiral ganglion cells) and the existing epileptic focus (fronto-precentral area), any seizure-provoking effect of cochlear field stimulation is uncertain. Thus, the fronto-precentral localization of the potentially epileptogenic focus in this case almost excludes the possibility of superimposed epilepsy of acoustic origin. In the present case, however, the intracochlear localization of all the 22 electrodes makes spread of impulses from the implanted stimulator exclusively to the acoustic nerve. Indeed, no additional peripheral nerve excitation was seen at either electrode programming protocol. These results seem to be in accordance with those of Gibbin *et al.* (1994) and Clark (1997) who did not find any complication with cochlear implantation of epileptic children on appropriate antiepileptic pharmacotherapy.

In summary, the results presented in this case report suggest that GM epilepsy *per se* is not a clinical contraindication for intracochlear implantation.

Acknowledgement

The authors express their gratitude to Ferenc Toth and Dr Katalin Avramov and Dr Peter Mayer for excellent technical assistance and consultations on special aspects of cochlear implantation in epilepsy, respectively. This work was supported by grants from the Hungarian National Health Foundation (OTKA) 6396 and 1353, Budapest, Hungary.

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Quarterly
ISSN 0001 - 6497
Vol. 52, 1998, 3rd trimester

ACTA OTO-RHINO-LARYNGOLOGICA BELGICA

Publication of the Royal Belgian Society for Ear, Nose, Throat, Head and Neck Surgery

Acta oto-rhino-laryngologica belg., 1998, 52, 253-256

Cochlear implantation in osteogenesis imperfecta

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Key words. Cochlear implantation ; osteogenesis imperfecta ; field stimulation

Abstract. *Cochlear implantation in osteogenesis imperfecta.* A case is reported in which a Nucleus 22 channel intracochlear implant was applied to a Hungarian woman (age 50 yr) with profound deafness associated with osteogenesis imperfecta. Successful intracochlear insertion of the 22 electrodes resulted in a 70 dB hearing improvement at frequencies 250-2000 HZ. Nevertheless, a characteristic facial twitching appeared upon activation of electrodes 9-13. Inactivation of these electrodes abolished the non-acoustic nerve excitation with preservation of acoustic performance. Osteogenesis imperfecta may involve a state of risk for non-acoustic nerve activation in cochlear implant patients possibly resulting from a reduced impedance to current spread by abnormal bone tissue. This, however can be overcome by simple programming manoeuvres.

Introduction

Osteogenesis imperfecta is a heritable defect that makes bones brittle because of a generalized osteopenia. The disease is frequently associated with progressive hearing loss usually beginning in the second decade of life and can be detected in 90 percent of subjects over age 30. The first association between hearing loss and osteogenesis imperfecta was noted as early as 1912 by ADAIR-DIGHTON. The loss can be conductive, sensorineural, or mixed and ranges widely in severity. In most cases, the middle ear usually exhibits maldevelopment, ossification abnormalities, sometimes abnormal calcium deposits (1). Therefore, we postulated that osteogenesis imperfecta patients, in whom the hearing loss derives predominantly from middle ear manifestation of the disease (often resembling those changes seen in otosclerosis), could benefit from intracochlear implantation. However, the abnormal bone structure of these patients may evoke difficulties during both surgery and the postoperative period. This paper draws attention to a special problem of non-acoustic nerve stimulation related to unique pathological changes in the temporal bone that occur in relation to osteogenesis imperfecta.

Case report

A 50-year old woman was referred to the cochlear implant unit of our department with a history of progressive hearing loss in both ears for many years. She had a history of established osteogenesis imperfecta. She had been totally deaf in her right ear for 19 years at the time of referral and could no longer benefit from conventional hearing aids on either side.

Pure tone audiometry yielded no response to air conduction stimuli up to *120* dB. No response was obtained on bone conduction at any frequencies to *80* dB in either ear. Auditory brainstem response using click stimuli was without effect in either ear. Promontory stimulation testing was carried out using the Nucleus promontory testing device and auditory percepts were obtained in both ears at

Fig. 1
High resolution CT scan of the temporal bone on side idented to implant. A radiographic picture resembling o otospongiotic bone deformity.

ll frequencies of stimulation from 50 Hz to 00 Hz with good dynamic ranges. Aided losed set speech recognition test revealed nvaluably low results. Computed tomography CT) scanning of the temporal bone was erformed on both sides. There was a loss of efinition of the cochlear architecture with emineralization and annular osteolysis in the urrounding otic capsule in either ear. The adiographic picture principally resembled that ypical of otosclerosis usually referred to as tospongiotic temporal bone deformity (Fig.). Nevertheless, the right ear seemed to be he less abnormal one with a preserved coch- ear lumen. Taking these findings together with he good results obtained with promontory timulation on this side, and after a discussion vith the patient of the possible gains to be xpected from cochlear implantation, a deci- ion was made to offer her an implant. The urgical procedure employed a standard pos- erior tympanotomy approach retaining the ncus. The ossicular chain exhibited a serious egenerative picture : joints of the incus with ne malleus and stapes were ossified to a severe egree making identification of the particular ones difficult. A separate cochleostomy was created in front of the round window niche and the scala tympani was entered. The electrode array passed into the cochlea without difficulty. All the 22 active electrodes were successfully positioned inside the cochlea. Intraoperative stimulation of the stapedius reflex was not successful due to metastatic calcification of the tendon of the stapedius muscle. Closure proceeded in the usual manner. Postoperatively there were no complications. A control X-ray performed on the second postoperative day confirmed the intracochlear localization of each electrode of the electrode array (Fig. 2).

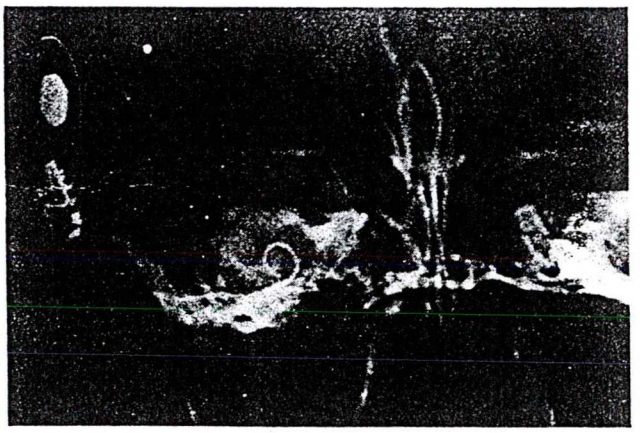

Fig. 2
Check X-ray control performed on the second postoperative day. It is confirmed that each electrode of the electrode array is intracochlear.

On the seventh post-operative day, a routine electrode check was carried out. Stimulation in common ground mode and bipolar + 1 mode produced facial twitching on electrodes 9-13. The channels to activate these electrodes were subsequently programmed out and as experienced by the patient subjectively and shown objectively by conventional audiology, a significant improvement in hearing was obtained with no evidence of further facial nerve activation (Fig. 3).

Discussion

To the best of our knowledge, this case report is the first example to show that hearing loss

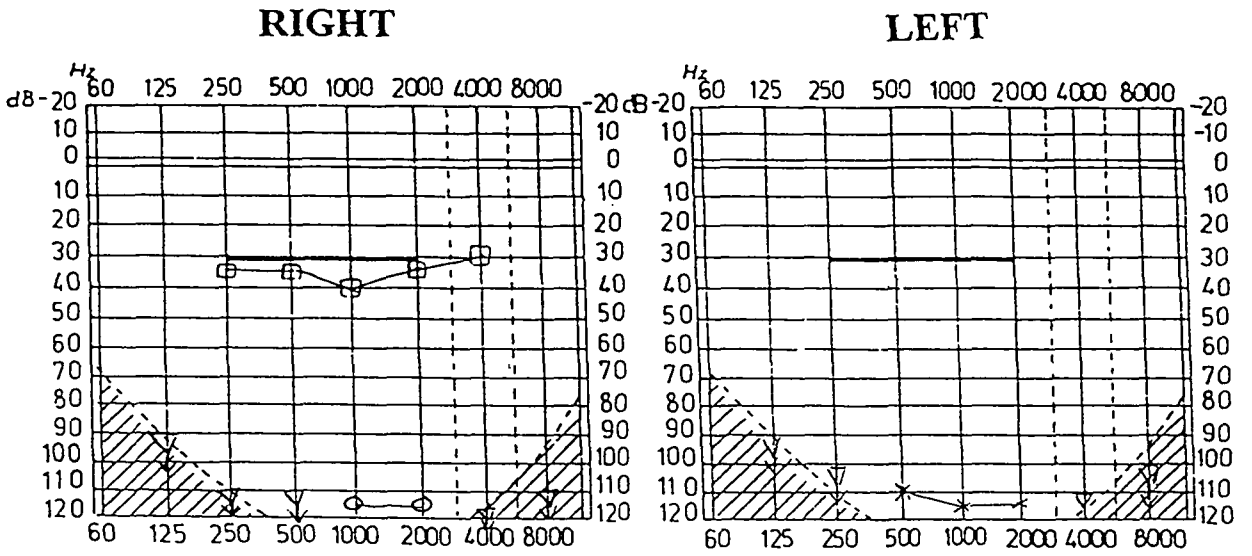

Fig. 3
Conventional audiology. A significant improvement in hearing is seen.
This was not accompanied by facial twitching after the electrodes 9-13 had been programmed out.

associated with osteogenesis imperfecta can be successfully treated with intracochlear implantation of a multichannel device. However, in spite of precise intracochlear positioning of each electrode of the electrode array of the Nucleus 22 equipment implanted, switching on of the device revealed facial twitching upon activation of electrodes 9-13.

Cochlear implantation has been shown to be a safe surgical procedure with few immediate or long-term complications (2). Unwanted effects include electrical stimulation of the facial nerve. In the absence of temporal bone abnormalities this adverse effect results mostly from current spread from electrodes which lie outside the cochlea (3), and if so, can be corrected by programming out the channels which stimulate non-acoustic neural elements. However, facial nerve stimulation sometimes results from current spread from electrodes positioned within the cochlea. Otosclerosis and temporal bone fracture are the two well-known conditions which are most likely to be associated with this phenomenon (4). However, a similar association between osteogenesis imperfecta and an increased likelihood for non-acoustic nerve excitation by intracochlear stimulation is not surprising, since temporal bone abnormalities in osteogenesis imperfecta and otosclerosis are very similar (5, 6). Moreover, Ross *et al.* (1993) postulated that otosclerosis and osteogenesis imperfecta in spite of differences in aetiology might sometimes coexist, otosclerosis being part of osteogenesis imperfecta. Thus, in osteogenesis imperfecta, a situation similar to that previously described in cochlear implant patients with temporal bone fracture or otosclerosis exists. That is, that intracochear stimulation may be fraught with danger. Regarding the mechanism it is possible that a decrease in electrical resistance in the diseased temporal bone due to either a line (after temporal bone fracture) or otospongiotic cavities (otosclerosis / osteogenesis imperfecta) may give rise to an electric field in the proximity of the facial nerve that results in a sufficient degree of depolarization of certain fibres. Nevertheless, no direct evidence has been provided for altered impedance of the otosclerotic / osteogenesis imperfecta temporal bone compared to normal bone.

Whatever the precise mechanism is, the present results clearly show that osteogenesis imperfecta similarly to otosclerosis, evokes a special clinical condition when electrical field

stimulation of the cochlea stimulates the facial nerve. Since electrical field stimulation activates all types of neurons in a corresponding electrical field with minimum or no selectivity for the neural elements it was intended originally to excite, we hypothesize that temporal bone abnormalities in this disease are possible, the underlying factors in the spread of the electrical field outside the cochlea. It is also observed that programming manoeuvres can overcome this adverse effect.

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Eur Arch Otorhinolaryngol (1997) 254 (Suppl. 1): S6–S8

BASIC SCIENCE

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J. Müller · J. Czigner

Involvement of glibenclamide-sensitive potassium channels in vasorelaxation by cochlear nerve stimulation

Abstract Rabbit aortic rings relaxed with an increase in cyclic guanosine monophosphate and cyclic adenosine monophosphate content in response to exposure to organ fluid of isolated cochleas of the guinea pig following field stimulation (50 Hz, 80 V, 0.2 ms). Relaxations were blocked by 30 μM N^G-nitro-L-arginine methyl ester added to the vessel rings. This inhibitory effect was reversed by 3 mM L-arginine. Removal of the vascular endothelium also blocked the relaxation response. Glibenclamide attenuated vasorelaxation in a concentration-dependent manner. We conclude that cochlear nerve stimulation induces an endothelium-dependent vasorelaxation involving activation of adenosine triphosphate-sensitive potassium channels.

Key words Cochlea · Field stimulation · Vascular endothelium · Vasorelaxation · Glibenclamide

Introduction

Acetylcholine, calcitonin gene-related peptide (CGRP) and nitric oxide (NO) have been found to play a neuromodulatory role in cochlear function [1, 2, 7, 10]. Since these neurotransmitters are known to induce vasodilation [6], we postulated that neurotransmitter release from cochlear nerves, due to acoustic and/or electric signals, might contribute to an increase in blood supply to the cochlea through vasodilation induced by these mediators. Since the vasodilatory effect of these mediators is believed to result at least in part from activation of adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}), the present study was devised to determine whether K_{ATP} activation was involved in the vascular effect of electrical activation of the isolated guinea pig cochlea.

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Materials and methods

Cochleas were prepared from pentobarbital (40 mg/kg)-anesthetized healthy adult guinea pigs and placed into an organ chamber containing Krebs bicarbonate buffer solution maintained at 37°C. Each 5 ml solution contained 118.1 mM NaCl, 4.7 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11.1 mM glucose. Solution was aerated with 95% O_2 and 5% CO_2 and kept at a pH of 7.4 ± 0.05.

To release neurotransmitters from the cochlea, field stimulation (FS) utilized 50 Hz, 80 V square impulses of 0.2 ms duration applied by means of an EXPERIMETRIA (London, England) programmable stimulator. In order to define the vascular effects of the neurotransmitters, 4-mm-long aortic rings were prepared from healthy male New Zealand rabbits weighing 3000–3500 g and tested for changes in isometric tension. The rings were allowed to equilibrate over 1 h at an initial tension of 20 millinewtons (mN). The preparations were then exposed to cumulative increases in phenylephrine concentrations followed by cumulative increases in acetylcholine concentration to test functional integrity of vascular endothelium [3]. After washout, the rings were precontracted with 1 μM phenylephrine and were then exposed to the organ fluid of the cochleas in which the FS had been accomplished.

Experiments were repeated with aortic rings having functionally intact endothelium and those from which the intimal surfaces had been removed. Rings with intact endothelium were tested for relaxation responses to the "cochlear fluid" in the presence of 30 μM N^G-nitro-L-arginine methyl ester (L-NAME) and successive incubation with 3 mM L-arginine (incubation period: 30 min for each). After washout, phenylephrine-precontracted aortic rings were exposed to the "cochlear fluid" in the presence of cumulative increases of glibenclamide and/or its vehicle concentrations. Finally, after washout, FS was repeated in the presence of 1 μM tetrodotoxin added to the cochleas.

Changes in vascular cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP) contents in response to exposure to the cochlear fluid at the above protocol were determined in separate rings using Amersham radioimmunoassay kits (Les Ulis, France) as described previously [8].

All data were expressed as means ± SD and were statistically analyzed by analysis of variance followed by the Bonferroni *t*-test. The level of significance was $P < 0.05$.

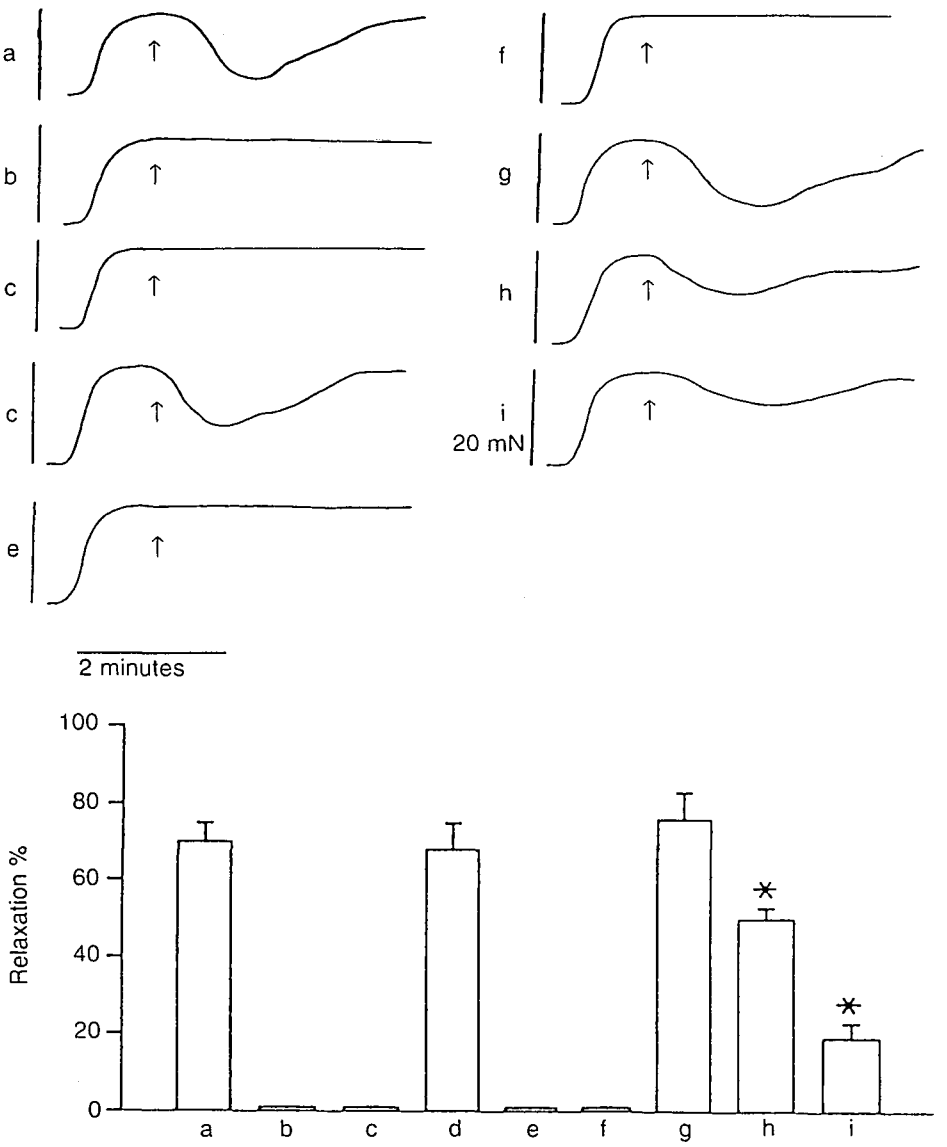

Fig. 1a–i Endothelium-dependent relaxation of rabbit thoracic aortic rings induced by exposure to "organ fluid" of isolated cochleas of the guinea pig after field stimulation. Original recordings indicating changes in tone of phenylephrine-precontracted aortic rings in response to exposure to organ fluid of isolated cochleas of the guinea pig after electrical field stimulation with 100 stimuli (50 Hz, 80 V, 0.2 ms). The *arrows* indicate commencement of exposure of the vessel rings to "cochlear fluid": a without treatment, b without preceding field stimulation, c after 30 µM N^G-nitro-L-arginine methyl ester (L-NAME) added to the organ fluid of the vessel rings, d after successive incubation with L-arginine (3 mM), e after removal of the vascular endothelium, f after 1 µM tetrodotoxin added to the organ fluid, g after 0.1 µM glibenclamide added to the medium of the vessel rings, h after 1 µM glibenclamide, i after 10 µM glibenclamide. Summarizing data obtained at the above protocols are presented below. Data are expressed as means ± standard deviation of the mean obtained from six experiments. * Significant difference between data obtained after exposure to the "cochlear fluid" without treatment and after glibenclamide at $P < 0.05$

Results

Phenylepinephrine-precontracted vessel rings relaxed in response to cochlear FS. The relaxation response was blocked by L-NAME when added to the organ chamber of the vascular preparation. In contrast, L-NAME added to the organ chamber of the cochlea did not influence FS-induced vascular relaxation. Additional incubation with L-arginine blocked the inhibitory effect of L-NAME. Removal of the vascular endothelium also blocked the relaxation response. Tetrodotoxin added to the cochlear fluid also blocked the effect of FS. Glibenclamide antagonized relaxation produced by the cochlear fluid (Fig. 1a–i). The solvent for glibenclamide (dimethyl-sulfoxide) was without effect.

Exposure to cochlear fluid after FS significantly increased levels of vascular cyclic GMP and AMP. Baseline values of either cyclic nucleotide did not change when rings were exposed to fluid of the non-stimulated cochleas

Table 1 Radioimmunoassay. Changes in rabbit aortic ring cyclic nucleotide content (pmol/mg wet tissue weight) by exposure to organ fluid (*CF*) of isolated guinea pig cochleas subjected to field stimulation (*FS*). The data are means ± standard deviation of the mean obtained from five experiments

Cyclic GMP			
Aortic rings	Control	CF without FS	CF with FS
Normal	0.081 ± 0.023	0.090 ± 0.019	0.15 ± 0.028*
Denuded	0.063 ± 0.025	0.079 ± 0.022	0.083 ± 0.029
Cyclic AMP			
Aortic rings	Control	CF without FS	CF with FS
Normal	0.285 ± 0.0253	0.278 ± 0.0343	0.718 ± 0.1358*
Denuded	0.199 ± 0.0261	0.183 ± 0.0297	0.248 ± 0.0639

*Significantly different from corresponding control values at $P < 0.05$

when FS was performed in the presence of 1 μM tetrodotoxin or when the effect of cochlear stimulation was tested on endothelium-free vessel rings (Table 1).

Discussion

The present results indicate that electrical FS of isolated guinea pig cochleas produces relaxation in rabbit aortic rings which is endothelium-dependent. In parallel with the relaxation response, a significant increase was found in both cyclic AMP and GMP contents in the aortic tissue. Relaxation was blocked by L-NAME, an inhibitor of NO synthase. Nevertheless, this inhibitory effect was reversed by additional incubation with L-arginine, indicating the relaxation response to be essentially nitrergic.

Glibenclamide, a specific blocker of K_{ATP} channels was found to attenuate the cochlear stimulation-induced relaxation of the rabbit aortic rings in a concentration-dependent manner, suggesting that K_{ATP} channel activation was involved in the relaxation mechanism. Since tetrodotoxin added to the cochlear preparation also blocked both the vascular response and changes in cyclic nucleotide content, our findings suggest that the currently undefined substance(s) from the electrically stimulated cochlea producing vasodilation are of neural origin.

Several neurotransmitters and neurohormones have been reported to exert at least some of their biological effect through activation of K_{ATP} channels. These include galanin, vasoactive intestinal polypeptide, CGRP, prostacyclin and norepinephrine [4]. These substances, however, share an ability to increase intracellular cyclic AMP level in target cells. An increase in cyclic AMP has been shown to be followed by K_{ATP} channel opening involving protein kinase A [4]. Nevertheless, any cyclic AMP elevating agent released from the cochlea could hardly be responsible for the vasorelaxation observed in our experiments, since such an agent would be expected to preserve its vasodilatory effect in the absence of an endothelial layer.

Many agents relax arterial smooth muscle through stimulation of the release of inhibitory factors from endothelial cells [3]. At least two endothelial factors are known to exert relaxation: NO, which was previously described as endothelium derived relaxing factor (EDRF) [5], and endothelium derived hyperpolarizing factor (EDHF) [9]. Both EDRF and EDHF can elicit hyperpolarization in vascular smooth muscle cells with the involvement of K_{ATP} channels. In our study, glibenclamide was found to attenuate endothelium-dependent vasorelaxation by our presumed cochlear neurotransmitters in a concentration-dependent manner. Nevertheless, since NO synthase inhibition completely blocked the relaxation response, we believe that NO-induced relaxation is only partially mediated by K_{ATP} channel activation.

It is widely accepted that activation of smooth muscle soluble guanylate cyclase by NO results in the accumulation of intracellular cyclic GMP, and produces a sequence of protein phosphorylation that leads to relaxation [5]. An increase in cyclic GMP levels might result in an increase in cyclic AMP to promote relaxation through inhibition of cyclic AMP breakdown by interaction between cyclic nucleotide phosphodiesterases. K_{ATP} channel opening seems to be profoundly involved in this mechanism, either due to a secondary increase in cyclic AMP, or by a direct cyclic GMP-regulated pathway. However, the lack of complete inhibition of cochlear stimulation-induced vasorelaxation by glibenclamide suggests either an incomplete blockade of these channels or the presence of an additional, K_{ATP}-independent relaxation mechanism by NO. Whatever the precise mechanism is, our present results seem to support the concept that the endothelial layer is a transducer of important vascular signals to which the vascular bed must respond in order to ensure adequate tissue perfusion according to moment-to-moment requirements. Our results also imply a possible beneficial effect of pharmacological maneuvers that utilize the NO→cyclic GMP pathway to support the clinical use of cochlear electrical stimulation.

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J Mol Cell Cardiol 27, 2559–2569 (1995)

The Loss of Pacing-induced Preconditioning in Atherosclerotic Rabbits: Role of Hypercholesterolaemia

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First Department of Medicine[1], Departments of Biochemistry[2], Endocrinology[3], Albert Szent-Györgyi University Medical School of Szeged, Hungary and Institut Henri Beaufour[4], Le Plessis Robinson, France

(Received 31 August 1994, accepted in revised form 19 June 1995)

Z. SZILVASSY, P. FERDINANDY, J. SZILVASSY, S. KARCSU, J. LONOVICS, L. DUX AND M. KOLTAI. The Loss of Pacing-induced Preconditioning in Atherosclerotic Rabbits: The Role of Hypercholesterolaemia. *Journal of Molecular and Cellular Cardiology* (1995) 27, 2559–2569. A brief rapid pacing has been shown to protect rabbit heart against global myocardial ischaemia induced by subsequent longer pacing. We studied whether pacing-induced preconditioning was reproducible in experimental hypercholesterolaemia. In conscious rabbits with an implanted right ventricular electrode and left ventricular polyethylene catheters, pacing of 500 bpm over 20 min induced an intracavitary ST-segment elevation of 3.2 ± 0.41 mV, shortened ventricular effective refractory period and increased left ventricular end-diastolic pressure from prepacing 105 ± 3.9 ms and 4.0 ± 0.93 mmHg to post-pacing 62 ± 6.4 ms and 27.9 ± 7.2 mmHg, respectively. A 10-min preconditioning pacing followed by a 5-min interval markedly attenuated these test pacing-induced ischaemic changes. Rabbits were fed a cholesterol-enriched diet over 4, 8 and 12 weeks, responded to a 5- or 10-min pacing with ischaemic changes of the same degree as did controls to a 10- or 20-min pacing, respectively. A 4-week diet elevated total serum cholesterol from 1.7 ± 0.4 to 24.1 ± 2.9 mmol/l without apparent atherosclerotic lesions in the thoracic aorta assessed by Oil-Red O staining and planimetry, but it abolished protection induced by a 5-min preconditioning pacing. A 12-week diet increased serum cholesterol and lesion surface area to 26.9 ± 3.2 mmol/l and $89.6 \pm 6.4\%$, respectively, and continued to block preconditioning. When these animals were refed normal chow over additional 6 weeks, serum cholesterol level dropped to 2.6 ± 0.80 mmol/l with no change in atherosclerotic lesions, the preconditioning effect, however, recovered. We conclude that hypercholesterolaemia blocks preconditioning irrespective of the development of atherosclerosis.

KEY WORDS: Ventricular overdrive pacing; Myocardial ischaemia; Preconditioning; Hypercholesterolaemia; Atherosclerosis.

Introduction

Single or repeated brief coronary artery occlusions render the heart resistant to a subsequent longer occlusion of the same artery (Murry *et al.*, 1986; Reimer *et al.*, 1986). The ability of the heart to adapt to repetitive ischaemic insults is termed as preconditioning (for review see Downey, 1992; Jennings and Reimer, 1991; Parratt, 1994). This endogenous mechanism has been shown to reduce infarct size, attenuate electrophysiological and haemodynamic changes due to regional or global myocardial ischaemia *in vivo* and *in vitro* (for review see Lawson and Downey, 1993). In addition, preconditioning induced by brief coronary artery occlusions has been reported to produce an anti-arrhythmic effect (Shiki and Hearse, 1987). Regarding the underlying mechanisms, the preconditioning insult has been suggested to promote the formation and release of some endogenous

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protective substances such as adenosine, bradykinin nitric oxide (NO) and prostacyclin that are supposed to mediate the anti-ischaemic and anti-arrhythmic effect (for review see Lawson and Downey, 1993). Whatever the proposed mechanism is, the great majority of animal studies reproducing the preconditioning phenomenon in rabbits, rats, dogs and pigs has been performed in anaesthetized, open-chest animals. Therefore, the results obtained are of limited pathophysiological significance, because of the confounding effects of anaesthetics, anaesthesia and the acute surgical intervention. However, very few recent studies revealed that the preconditioning effect could be observed in conscious rabbits subjected to repeated brief periods of coronary artery occlusion (Cohen *et al.*, 1994) or rapid ventricular overdrive pacing (VOP) to induce myocardial ischaemia (Szilvassy *et al.*, 1994a). Nevertheless, these studies have been performed with healthy animals demonstrating that the *in situ* or *in vitro* normal heart is able to adapt to repetitive ischaemic challenges.

Hyperlipidaemia and atherosclerosis and their major clinical sequel, the ischaemic heart disease, constitute the leading cause of death in modern society. The heart of atherosclerotic patients is hardly capable of adapting to physical exercise and other kinds of stress. The present study was undertaken to examine whether an impaired capability of the *in situ* heart to adapt to repeated ischaemic insults was involved in the mechanisms responsible for the increased severity of ischaemic attacks in conscious rabbits with hyperlipidaemia and atherosclerosis induced by exposure to a cholesterol-enriched diet. The results presented here suggest that the preconditioning phenomenon is lost with the development of experimental hypercholesterolaemia and atherosclerosis in conscious rabbits subjected to global myocardial ischaemia induced by VOP. It is also suggested that hypercholesterolaemia *per se* rather than atherosclerosis is responsible for the loss of preconditioning. A part of this study was presented at the Annual Meeting of the European Section of International Society for Heart Research (Szilvassy *et al.*, 1994b).

Materials and Methods

Animals and surgery

The experiments were carried out with 40 adult, male New Zealand white rabbits, weighing 3.0–3.5 kg, housed as described (Szilvassy *et al.*, 1993, 1994a). The animals were fed commercial laboratory chow and tap water *ad libitum*. Surgery was performed under aseptic conditions after a 2-week adaptation period. The rabbits were anaesthetized with an intravenous bolus of diazepam (Sigma, St. Louis, MO, USA, 10 mg/kg body weight) and ketamine (EGIS Pharmaceuticals, Budapest, Hungary, 5 mg/kg); lidocaine (EGIS Pharmaceuticals, Budapest, Hungary, 10mg/kg) was given for local pain relief. A bipolar French four-electrode catheter (Cordis, Erkrath, Germany) was introduced into the right ventricle through the main branch of the right jugular vein to obtain the intracavitary electrogram. The correct position of the catheter was confirmed by analysing the intracavitary electrogram as described (Szilvassy *et al.*, 1993). Almost the whole catheter was fixed under the skin of the neck making a subcutaneous cradle for the precurved device. The proximal part of a 4-mm length of the electrode was exteriorized through a separate incision. During the experiments, this part of the catheter was fitted to a two channel programmable stimulator (Experimetria, UK) through a special connector for French four catheters (Cordis, Erkrath, Germany). A polyethylene cannula was inserted into the left ventricular cavity through the right external carotid artery to measure left ventricular pressure. The proximal part of the cannula was exteriorized through a separate incision and connected to an "Experimetria UK" two channel electromanometer through a Statham P23 Db Transducer (Gould, Balainviliers, France); another polyethylene cannula was introduced into the distal third of the central ear artery for recording mean arterial blood pressure (MABP). This latter cannula was connected to the second channel of the electromanometer through a separate Statham transducer. The volumes of both cannulas were precisely determined prior to surgery, and each cannula was then filled with appropriate volume of Na-heparin (5000 IU/ml, Richter Pharmaceuticals, Budapest, Hungary) to prevent blood coagulation in the cannulas. Because of the soft surgery technique, no systemic antibiotic treatment was necessary, nevertheless, 10^6 IU penicillin (Zyma-Biogal Pharmaceuticals, Debrecen, Hungary) was applied locally prior to closing the neck wound. A 7-day period of convalescence was allowed for each animal before commencement of the experiments.

Cardiac electrophysiology and haemodynamics

The right intracavitary electrogram, the chest lead ECG, the left ventricular pressure and MABP were

continuously recorded by means of an Experimetria UK six-channel multiscriptor. Right ventricular effective refractory period (VERP) was determined as described (Szilvassy *et al.*, 1993). In brief, electrical square impulses of 1.5 ms duration at twice the diastolic threshold were delivered through the implanted electrode by means of the programmable stimulator. Single programmed extrastimuli were introduced late in diastole following 15 basic driven beats of 20 ms cycle length. The coupling interval of the programmed stimuli were then gradually shortened in 2-ms steps until disappearance of signs of ventricular activation in the chest-lead ECG. The longest coupling interval that failed to produce activation of the ventricles was referred to as VERP.

Global myocardial ischaemia induced by ventricular overdrive pacing

The induction of global myocardial ischaemia was based on a method described elsewhere (Szilvassy *et al.*, 1994a). Conscious rabbits were subjected to VOP at 500 bpm with double threshold square impulses of 1.5 ms duration over 5, 10 and 20 min. The resulting (post-pacing) right intracavitary ST-segment elevation, increase in LVEDP (Fig. 1) and shortening of VERP were measured to characterize the severity of ischaemia. Post-pacing ST-segment elevation was determined 40 ms after the "J"-points. LVEDP was determined after the "A" wave immediately prior to the first phase of left ventricular pressure upstroke appearing simultaneously with the appearance of the "S" wave in the chest lead ECG. The "S" wave in the chest lead ECG was used as a reference point for LVEDP determination to assure that the same point in the cardiac cycle was examined in each case. Mean values of five consecutive cardiac cycles before and after VOPs produced the data for evaluation. VERP was measured under resting conditions (before VOP) and 20–30 s following cessation of VOP (post-pacing values).

Histomorphometry and vascular reactivity

For measurement of aortic lesion surface area, the thoracic aorta (from the left subclavian artery to the diaphragm was removed from each animal at autopsy. The vessels were dissected free of the adventitial tissue and any remaining blood was rinsed clean. The aortae (except the distal parts used for vascular reactivity studies) were dissected

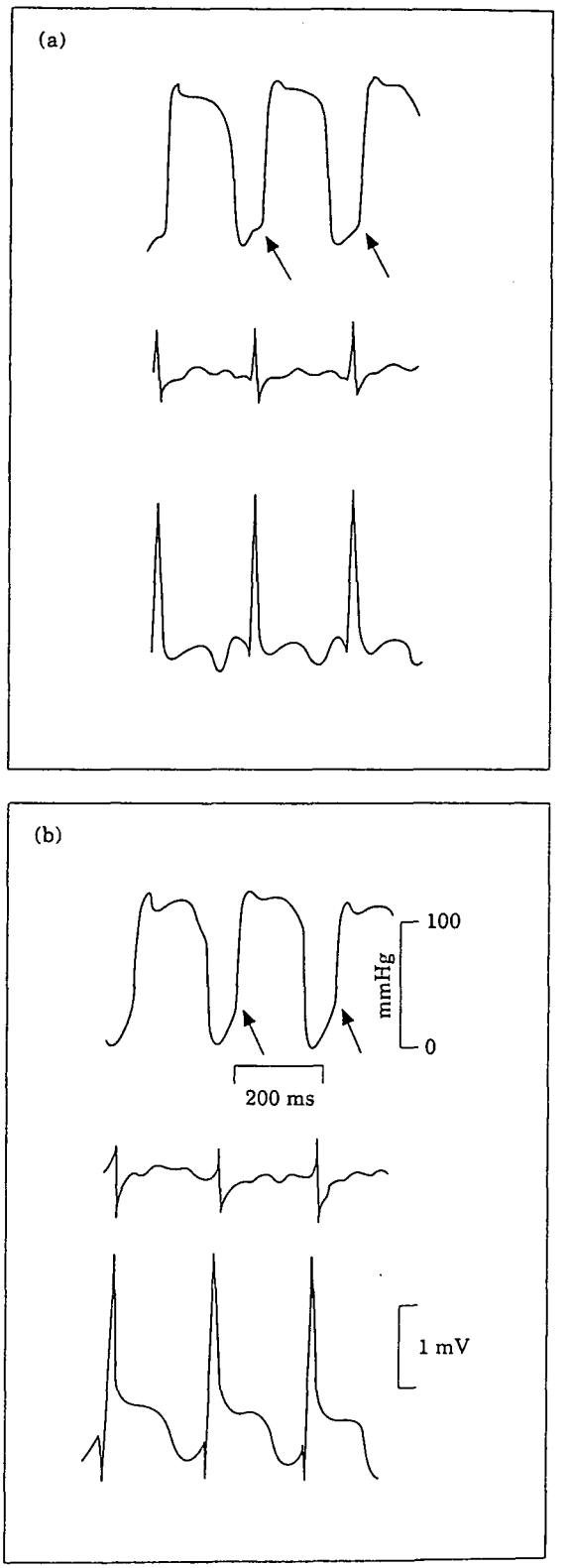

Figure 1(a) Original recording of the left ventricular pressure curve (top tracing), the chest lead ECG (middle) and the right intracavitary electrogram (bottom tracing) under resting conditions (pre-pacing values) in conscious rabbits. The arrows show the points of determination of the left ventricular end-diastolic pressure (LVEDP) in consecutive cardiac cycles. (b) Original recording showing the effect of a single period of ventricular overdrive (500 bpm over 5 min) on the LVEDP (top) the chest lead ECG and the intracavitary electrogram (post-pacing values).

longitudinally and placed on a glass plate for photography of intimal lesions before and after staining in Oil-Red O dissolved in isopropyl alcohol (0.3% wt/vol in 60% vol/vol), for 5 min, rinsed in 60% isopropyl alcohol, then stained for 1 min in Harris' haematoxylin solution. Lesion surface area and total vessel surface area were measured by planimetry of the photographic images. Lesion surface area was expressed as mean percentage of the total surface area.

The distal parts of the thoracic aortae were immersed in oxygenized Krebs solution containing in mM: NaCl: 118.1, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.0, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1, gassed with 95% O_2 and 5% CO_2 at 37°C. The vessel parts were cleaned of fat and adhering connective tissue, then vessel rings of 4 mm length were prepared. The rings were mounted horizontally on two small L-shaped glass hooks of which one was connected to a force transducer (SG-2, Experimetria, Budapest, Hungary) for measurement and recording of isometric tension. The experiments were carried out in an organ bath of 5 ml volume. The vascular endothelium was preserved during preparation. Norepinephrine bitartarate (NE) and acetylcholine chloride (ACh, Sigma, St Louis, MO, USA) were dissolved in distilled water: their concentration referred to the base. The initial resting tension was set at 20 mN, and the preparations were allowed to equilibrate for 60 min. Subsequently, the vessel rings were exposed to increasing concentrations of NE added to the organ bath in 50 μl volume at half log increments in a cumulative manner. After the maximum contractile response to NE was obtained, the preparations were rinsed with Krebs solution until tension returned to previous baseline level. To study the vessel rings for endothelium-dependent relaxation, the rings were precontracted EC_{50} concentration of NE. After a stable contraction was attained, the preparations were exposed to increasing concentrations of ACh in half log increments in a cumulative manner. ACh concentrations that induced significant relaxation of the NE-precontracted aortic rings with intact endothelium, failed to elicit any relaxation response after removal of the endothelial layer (data not shown).

Experimental protocol

On the 7th day of convalescence after surgery, the animals were randomly divided into two groups.

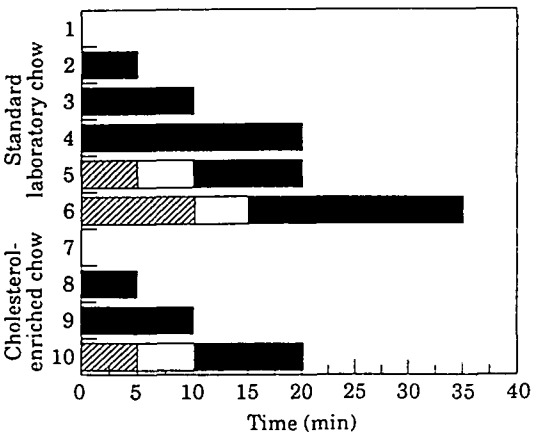

Figure 2 Pacing protocols in groups of conscious rabbits: (1) = without pacing, (2) = after a single 5-min, (3) = 10-min and (4) = 20-min pacing, (5) = 10-min test pacing preceded by a 5-min preconditioning pacing, (6) = 20-min test pacing preceded by a 10-min preconditioning pacing in rabbits fed standard laboratory chow; (7) = without pacing, (8) = after a single 5-min, (9) = 10-min pacing and (10) = 10-min test pacing preceded by a 5-min preconditioning pacing in rabbits fed cholesterol-enriched diet; duration of preconditioning pacing ▨; interpacing interval □; duration of test pacing ■.

Eight animals were continued to feed ordinary laboratory chow (control group), whereas the other 32 rabbits were started to feed laboratory chow enriched with 1.5% cholesterol (experimental groups) over periods of four (eight animals), eight (eight animals) and 12 weeks (16 animals). Eight animals from the "12-week" experimental group were refed normal chow over additional 6 weeks (regression group). Serum cholesterol level was measured after the 7-day period of convalescence and at the end of corresponding periods of cholesterol-enriched diet by means of an automatic analyser (Beckman Model 700 Chemistry System, Miami, FL, USA) using Boehringer cholesterol kits (Ingelheim, Germany). To control liver function, serum bilirubin was assayed at corresponding time points.

Figure 2 shows the pacing protocols used to assess electrophysiological and haemodynamic changes produced by single periods of VOP of 5, 10 and 20 min duration in rabbits fed normal chow and those used with animals fed cholesterol-enriched diet i.e., VOPs over 5 and 10 min. VOP of 20 min duration could not be applied in the hypercolesterolaemic groups, since these animals frequently develop repetitive ventricular extrasystoles in response to VOP over 20 min, which made electrophysiological and haemodynamic measurements incorrect. To characterize the severity of myocardial ischaemia,

induced by single periods of VOP. VERP and LVEDP were determined prior to and after cessation of VOPs as described above. Post-pacing intracavitary ST-segment elevation was also determined after each pacing period. To study the effect of a preceding brief period of pacing on ischaemic changes produced by a subsequent one, i.e. VOP-induced preconditioning, a 5-min or 10-min preconditioning VOP (PC VOP) of 500 bpm was applied prior to the 10-min or 20-min "test" pacing with an interpacing interval of 5 min in healthy animals. In hypercholesterolaemic animals and in the "regression group", 5-min pacing (500 bpm) was used to induce preconditioning and a subsequent 10-min period of VOP (500 bpm) was used to test the preconditioning effect. The interpacing interval was of 5 min duration, again. Each pacing protocol was repeated twice divided by a 48-h interval, to avoid any interaction between consecutive ischaemic challenges. Determinations were performed: prior to exposure to a cholesterol-enriched diet (0 time); at the end of 4-, 8-, 12-week periods of exposure to cholesterol-enriched diet and after 18 weeks in the "regression group". Two animals per group were excluded from the study because of the mechanical damage of the implanted catheters/connectors or due to other accidental problems, thus six rabbits per group were selected for measurements. Following completion of *in vivo* experiments (electrophysiological and haemodynamic determinations), each animal was killed, then the thoracic aortae were harvested for histomorphometry and vascular reactivity studies.

Statistical analysis

All data are expressed as mean ± standard deviation (S.D.). Electrophysiological and haemodynamic parameters were statistically analysed by ANOVA followed by a modified *t*-test for unpaired data. *P* values were adjusted according to the Bonferroni's method (Wallenstein *et al.*, 1980). In vascular reactivity studies, concentration–effect curves were constructed by expressing contractions as a percentage of the maximal response to NE; relaxations by ACh were expressed as a percentage of the tension induced by exposure to EC_{50} concentration of NE. Multiple comparisons were avoided by comparing EC_{50} and maximal responses only. Comparisons among means obtained from six measurements were analysed by the Student's *t*-test. Histomorphometric data were given as percentage of the atheromatous lesion of the total surface area of thoracic aortae. Differences were considered significant at $P<0.05$.

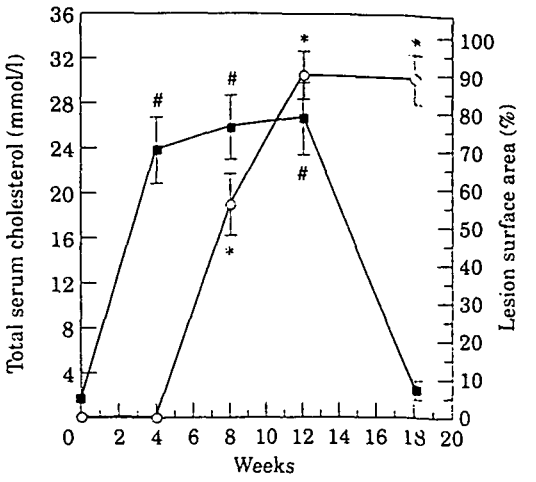

Figure 3 Line plots of total serum cholesterol (○) and lesion surface area (■) in rabbits fed standard laboratory chow (at 0 time), cholesterol-enriched diet over 4, 8 and 12 weeks and when a 12-week cholesterol-enriched diet was followed by standard laboratory chow for additional 6 weeks. Results are means ± S.D. obtained with six rabbits. # significant increase in serum cholesterol: * significant increase in lesion surface area. $P<0.05$.

Results

Laboratory measures, vascular histomorphometry and reactivity

In the course of the cholesterol-enriched diet, total serum cholesterol, bilirubin, vascular morphological lesions and ACh-induced endothelium-dependent vasodilation in isolated thoracic aortic rings were followed. Atherosclerosis was considered if lesions were visible on the aortic endothelial surface and the vasodilator effect of ACh was impaired. After 4, 8 and 12 weeks of a cholesterol-enriched diet, serum cholesterol increased from 1.7 ± 0.4 to 24.1 ± 2.9, 26.0 ± 2.8 and 26.8 ± 3.2 mmol/l, whereas it dropped to 2.6 ± 0.8 mmol/l in the "regression group" at 18 weeks (Fig. 3). Serum bilirubin was 7.7 ± 2.6 μmol/l in healthy rabbits and yielded concentrations of 7.4 ± 3.9, 9.2 ± 3.1 or 11.0 ± 4.4 μmol/l at the end of the 4th, 8th or 12th week, respectively. Changes in serum bilirubin proved to be statistically not significant when compared with control.

The thoracic aortae excised from animals fed normal laboratory chow or a cholesterol-enriched

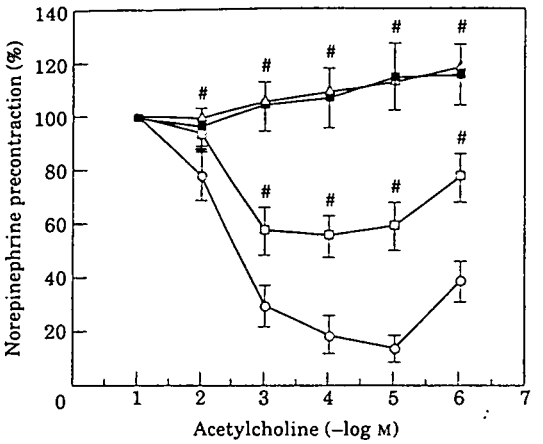

Figure 4 Acetylcholine-induced endothelium-dependent vasodilation in norepinephrine-precontracted thoracic aortic rings isolated from rabbits fed normal chow (control group; (○) and cholesterol-enriched chow over 8 (□) and 12 weeks (△), and when a 12-week cholesterol-enriched diet was followed by standard laboratory chow for addition 6 weeks = regression group (■). Results are means ± S.D. obtained with six rings in each group. # significantly different from corresponding values in the control group. Level of significance: $P<0.05$.

diet over 4 weeks showed no evidence for atheromatous lesions by visual inspection with or without Oil-Red O staining. Planimetric analysis by an unbiased histologist revealed that after periods of an 8- or 12-week cholesterol-enriched diet, lesion surface areas were 55.8 ± 8.0 or 89.6 ± 6.4% of the total surface, respectively (Fig. 3). When the dietary cholesterol overload was terminated and the rabbits were continued to feed standard laboratory chow ("regression group"), aortic atheromatous lesions exhibited no tendency for recovery. In aortic rings isolated from healthy rabbits, the maximal increase in isometric tension and the $-\log EC_{50}$ of NE were 21.1 ± 2.4 mN and 6.8 ± 0.1, respectively, and were not changed by a cholesterol-enriched diet. The maximum relaxation induced by ACh was 92.0 ± 5.6% in rings of rabbits fed standard laboratory chow (Fig. 4), and showed no significant impairment after a 4-week exposure to cholesterol overload (90.0 ± 5.1%, data not shown in Fig. 4). There was a marked and statistically significant decrease in endothelium-dependent vasodilation after an 8-week cholesterol-enriched diet, whereas a 12-week diet abolished ACh-induced relaxation, and the rings were slightly but concentration-dependently contracted by ACh. When the cholesterol-enriched chow was replaced by standard laboratory chow for additional 6 weeks, the aortic rings remained unresponsive to the relaxing effect of ACh.

Myocardial ischaemia in normal and hypercholesterolaemic rabbits

VOP-induced global myocardial ischaemia was characterized by right ventricular intracavitary ST-segment elevation, VERP shortening and LVEDP increase. In contrast to the right intracavitary electrogram, VOP produced no detectable ST-segment elevation/depression in the chest lead ECG. Table 1 shows ST-segment elevation, shortening of VERP and increase in LVEDP produced by VOP of 500 bpm over 5, 10 and 20 min in rabbits fed standard laboratory chow and VOP of the same rate over 5 and 10 min in animals fed a cholesterol-enriched diet over 4, 8 and 12 weeks. The results show that in normal rabbits the severity of intracavitary ST-segment elevation was proportional to the duration of VOP. The overall follow-up of VOP-induced ST-segment elevation in rabbits fed cholesterol-enriched diet or in the "regression group" showed that VOP of standardized rate and duration produced more severe intracavitary ST-segment changes than in the control group except values measured after the 10-min VOP in the "4-week" group. Resting VERP tended to be shorter in the hypercholesterolaemic/atherosclerotic animals, however, this did not reach the required level of significance (Table 1). All post-pacing VERP values were significantly shorter as compared to the prepacing ones, however, no significant changes were noted when control values were compared to those obtained in hypercholesterolaemic/atherosclerotic animals. VOP induced a significant increase in LVEDP as compared to resting values (Table 1). Cholesterol-enriched diet of longer exposure period than 4 weeks significantly increased resting LVEDP apparently as a function of the duration of the cholesterol overload. Post-pacing LVEDP-increase was also significantly greater in groups exhibiting high serum cholesterol and consecutive atherosclerosis. None of these changes returned to normal when rabbits were refed standard laboratory chow over additional 6 weeks ("regression group").

In healthy rabbits, resting MABP was 88 ± 3.9 mmHg, whereas it increased to 93 ± 4.1 mmHg ($P>0.05$) after 4-week exposure to a cholesterol-enriched diet. However, after 8 or 12 weeks of excess dietary cholesterol intake, MABP increased up to 106 ± 4.9 and 115 ± 6.8 mmHg, respectively ($P<0.05$). When a cholesterol-enriched diet was replaced by standard laboratory chow,

Table 1 Intracavitary ST-segment elevation, shortening of ventricular effective refractory period and increase in left ventricular end-diastolic pressure induced by single periods of VOP in rabbits fed standard laboratory chow and cholesterol-enriched diet.

Groups	Duration of diet (weeks)	Duration of VOP (min)	ST-segment elevation (mV)	VERP (ms)		LVEDP (mmHg)	
				Resting	Post-pacing	Resting	Post-pacing
Control diet	0	5	1.2±0.07	106±3.1	87±2.6#	4.1±0·79	10.7±2.60#
	0	10	2.2±0.22+	102±3.7	78±3.0#	3.9±0.74	13.1±3.00#
	0	20	3.2±0.41+	105±3.9	62±6.4	4.0±0.93	27.9±7.20#
Cholesterol-enriched diet	4	5	1.9±0.21+	101±3.6	82±4.3#	5.1±1.65	14.8±3.55#
	4	10	2.5±0.35+	105±4.4	71±3.8#	5.0±1.40	20.0±4.60#
	8	5	2.45±0.45+	103±5.0	79±3.7#	9.2±2.60*	17.2±5.73#
	8	10	2.9±0.35+	98±5.2	70±6.4#	9.6±2.00*	24.3±4.82#
	12	5	2.5±0.49+	99±4.2	78±3.7#	11.0±2.9*	19.9±4.68#
	12	10	3.0±0.37+	99±5.6	70±6.2#	13.0±2.5*	28.0±2.70#
Cholesterol-enriched diet + normal diet	12+6	5	1.9±0.29+	101±4.0	88±4.9#	12.0±3.80*	18.2±4.00#
	12+6	10	2.9±0.30+	99±5.9	71±4.1#	13.0+2.50*	28.0+2.70#

VOP = ventricular overdrive pacing, VERP = right ventricular effective refractory period. LVEDP = left ventricular end-diastolic pressure. Results are means ± S.D. obtained with six rabbits. +statistically significant difference in ST-segment elevation as compared to that induced by a 5-min VOP in healthy rabbits. # significant difference between resting and post-pacing values. * significant difference between resting LVEDP in rabbits fed normal, cholesterol-enriched diet or cholesterol-enriched diet + standard laboratory chow.

MABP decreased slightly to 107 ± 5.0 mmHg, but still remained significantly higher than the control value. Resting heart rate of normal rabbits was in the range of 230–270 bpm, and it was not changed by the cholesterol-enriched diet.

Preconditioning in normal and hypercholesterolaemic rabbits

In rabbits fed standard laboratory chow, a 5-min or 10-min PC VOP, performed 5 min prior to a 10-min or 20-min test VOP, markedly attenuated intracavitary ST-segment elevation (Fig. 5). VERP shortening (Fig. 6) and LVEDP increase (Fig. 7), indicating a decrease in severity of global myocardial ischaemia induced by the test VOPs. In contrast, when groups of rabbits were fed a cholesterol-enriched diet over 4, 8 and 12 weeks, a 5-min PC VOP did not significantly alter ST-segment elevation, VERP shortening and LVEDP increase provoked by a 10-min test VOP (Figs 5, 6 and 7) showing that preconditioning induced by a preceding shorter period of VOP became ineffective to protect the heart against electrophysiological and haemodynamic consequences of global myocardial ischaemia produced by the test VOP. On the other hand, in the "regression group", in parallel with the dramatic decrease in total serum cholesterol (Fig. 3), a 5-min PC VOP again markedly reduced ST-segment elevation (Fig. 5), VERP shortening (Fig. 6) and LVEDP increase (Fig. 7) provoked by the 10-min test VOP.

Discussion

In agreement with our previous findings (Szilvassy *et al.*, 1994a), the results presented here show that in healthy, conscious rabbits a preceding single brief period of VOP attenuates intracavitary ST-segment elevation, shortening of VERP and increase in LVEDP resulting from global myocardial ischaemia induced by a subsequent longer period of VOP. These results also demonstrate that this protection is lost when hypercholesterolaemia either with or without consecutive atherosclerosis develops. On the other hand, the ability of the *in situ* rabbit heart to adapt to repetitive pacing-induced ischaemic insult is restored in parallel with renormalization of the serum cholesterol level in spite of the presence of severe atherosclerotic lesions. In the course of a cholesterol-enriched diet, rabbits responded with more marked global myocardial ischaemia to standardized single brief periods of VOP than did animals in the control group. Intracavitary ST-segment elevation induced by a 5-min VOP was significantly

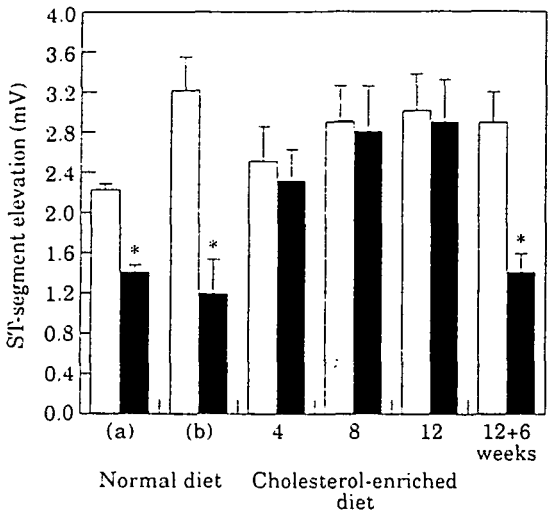

Figure 5 Effect of a preconditioning ventricular overdrive pacing on test pacing-induced intracavitary ST-segment elevation in rabbits fed normal laboratory chow and cholesterol-enriched diet. In the "12 + 6 group" the animals were fed cholesterol-enriched diet over 12 weeks, then they were continued to feed normal chow over additional 6 weeks. The effects of single test pacings (without preconditioning □): effect of test pacing preceded by preconditioning pacing with a 5-min interpacing period (■). In controls (normal diet), a 10-min (first open column) or 20-min (second open column) test pacing preceded by a 5-min or 10-min preconditioning pacing, respectively, was applied, whereas in the other groups a 10-min pacing was used for "test" pacing and a 5-min pacing was used to induce preconditioning with an interpacing interval of 5 min. Results are means ± S.D. obtained with six animals. * significant difference between non-preconditioned and preconditioned values. $P<0.05$.

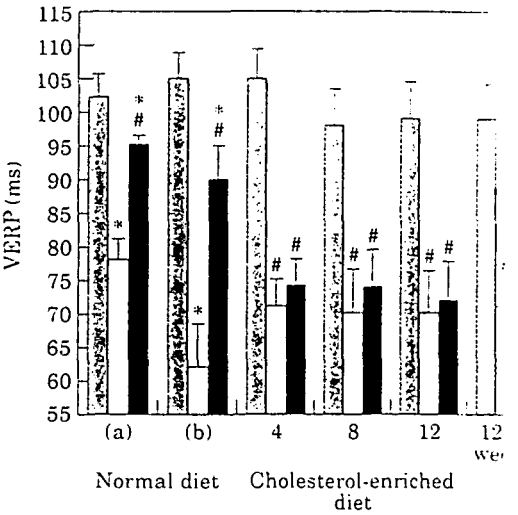

Figure 6 Effect of preconditioning ventricular ove pacing on test pacing-induced shortening of ventr effective refractory period in rabbits fed normal cholesterol-enriched diet. In the group of 12 + 6 r were fed cholesterol-enriched diet over 12 weeks continued to feed normal diet for additional 6 w Resting i.e. baseline values (▨). Post-pacing values ured after single periods of test pacing (□). Post-p values measured when a test pacing was preceded preconditioning pacing (■). In controls (normal d 10-min or 20-min test pacing and a 5-min or 1 preconditioning pacing were applied whereas in the groups a 10-min test pacing and a 5-min preconditi pacing was used. Results are means ± S.D. obtained six animals. *significant difference between non conditioned and preconditioned hearts. ^significan ference between corresponding resting and post-p and #normal and atherosclerosis resting values. $P<$

greater in the hypercholesterolaemic groups as early as 4 weeks after commencement of dietary cholesterol overload. When hypercholesterolaemia led to progressive atherosclerosis, ST-segment elevation became significantly more marked in all groups fed cholesterol-enriched diet, and this alteration remained characteristic of rabbits whose serum cholesterol dropped near the normal level resulting from refeeding normal laboratory chow. Another sensitive marker of global myocardial ischaemia, the post-pacing increase in LVEDP changed in a similar manner during atherogenesis. Resting LVEDP showed a tendency to increase even after a 4-week period of cholesterol overload, however, when atherosclerosis became apparent, it was significantly increased as compared to control values. VOP markedly increased LVEDP in all groups studied, and this post-pacing increase was also significantly augmented at various stages of dietary cholesterol overload, especially after the manifestation of atherosclerosis. In no cholesterolaemic/atherosclerotic rabbits thes terations in LVEDP were still present. VERP considerably shortened by global myocardial i aemia provoked by single periods of VOP eith normal rabbits or in those subjected to die cholesterol overload. Both the resting and pacing values showed a tendency to shorten result of hypercholesterolaemia and atheroscler the differences were, however, not statistically nificant when compared with control.

MABP slightly increased at the end of the week period of cholesterol-enriched diet and also significantly higher than normal after a week or 12-week cholesterol overload. In ac with ST-segment elevation and LVEDP increase elevation of MABP was present when the erosclerotic animals were re-exposed to stan laboratory chow. Elevated MABP might at lea part explain the increase in resting and post-pa LVEDP in hypercholesterolaemic/atheroscler

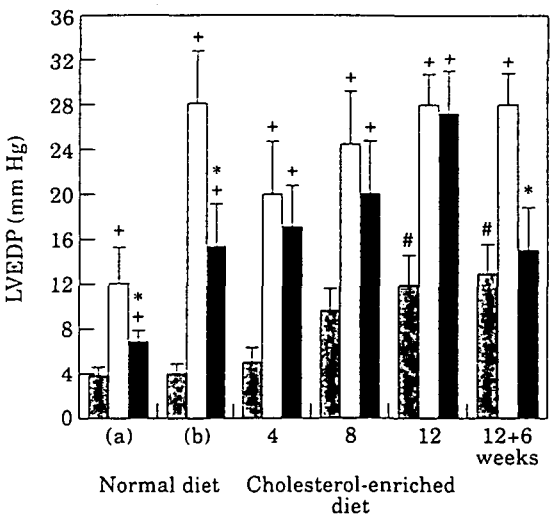

Figure 7 Effect of a preconditioning ventricular overdrive pacing on test pacing-induced increase in left ventricular end-diastolic pressure in rabbits fed normal and cholesterol-enriched diet. In the group of 12 + 6 rabbits were fed cholesterol-enriched diet over twelve weeks then continued to feed normal diet for additional 6 weeks. Resting i.e. baseline values (■). Post-pacing values measured after single periods of test pacing (□). Post-pacing values when a test pacing was preceded by a preconditioning pacing (■). In controls (normal diet) a 10-min or 20-min test pacing and a 5-min or 10-min preconditioning pacing were applied, whereas in the other groups a 10-min test pacing and a 5-min preconditioning pacing was used. Results are means ± S.D. obtained with six animals. * significant difference between non-preconditioned and preconditioned values. + significant difference between corresponding resting and post-pacing and # normal and atherosclerotic resting values. $P<0.05$.

rabbits. In conclusion, the severity of VOP-induced global myocardial ischaemia and the elevation of MABP appear to correlate better with the extent of atherosclerotic alterations than with an increase in serum cholesterol level. These alterations mimic the symptoms that are characteristic of atherosclerotic clinical patients.

The major finding of this study shows that the loss of VOP-induced preconditioning is due to an elevated serum cholesterol level attained by a 4-week exposure to a cholesterol-enriched diet. As measured by Oil-Red O staining and planimetry as well as by *ex vivo* ACh-induced endothelium-dependent vasodilation, this period of cholesterol overload neither resulted in any visible damage of the aortic endothelium, nor impaired its function. The conclusion may therefore be drawn that preconditioning is lost at a very early stage of atherogenesis, even before the development of any morphological and functional alterations in large conductance vessels. Preconditioning is continuously blocked during a longer exposure of rabbits to cholesterol-enriched diet, which markedly reduced the intact endothelial surface of the thoracic aorta with a consecutive impairment of ACh-induced endothelium-dependent vasodilation. Nevertheless, normalization of serum cholesterol level following termination of dietary cholesterol overload in rabbits fed a cholesterol-enriched diet over 12 weeks leads to the recovery of the preconditioning effect regardless the presence of vascular atherosclerotic alterations.

Shiki and Hearse (1987) were the first who provided evidence for the anti-arrhythmic effect of ischaemic preconditioning in isolated rat hearts. Our recent experiments with conscious rabbits have revealed that VOP-induced preconditioning protects the *in situ* rabbit heart against shortening of VERP due to myocardial ischaemia (Szilvassy *et al.*, 1994a). Reduction of ventricular refractoriness is thought to be one of the crucial factors underlying ischaemia/reperfusion-induced arrhythmias. Our findings therefore seem to support the hypothesis that preconditioning protects against arrhythmias through interacting with baseline mechanisms of arrhythmogenesis. The ischaemia-induced VERP reduction is believed to reflect the shortening of action potential duration by opening of ATP-sensitive potassium (K_{ATP}) channels (Noma, 1983). These channels have been claimed to contribute to the cardioprotective effect of ischaemic preconditioning in the *in situ* dog heart through activation of adenosine A_1 receptors (Gross and Auchampach, 1992). The opening of the K_{ATP} channel is supposed to confer a cardioprotective effect since it reduces action potential duration which translates to less time spent in contraction as a result of sparing ATP and reducing calcium entry. At the same time, however, opening of the K_{ATP} channel promotes the generation of re-entry-type ventricular arrhythmias.

The contradiction between the finding of Gross and Auchampach (1992) and our results in terms of the relative prolongation by preconditioning of ventricular refractoriness may be explained by differences in species (dog *v* rabbit) and experimental conditions used (anesthaesia, acute surgery *v* conscious stage, chronic instrumentation). The explanation for the loss of VOP-induced preconditioning in hypercholesterolaemic rabbits is obscure. The close correlation between the increase in serum cholesterol and the abolition of the cardioprotective effect conferred by preconditioning suggests that hypercholesterolaemia and/or hyperlipidaemia may impair myocardial function

through a direct deteriorating effect of serum lipids on the heart. This may account for the decreased tolerance of hypercholesterolaemic/atherosclerotic hearts to repetitive ischaemic insults. Evidence has been presented that hypercholesterolaemia can enhance endothelium-derived superoxide anion production (Ohara *et al.*, 1993). Increased free radical formation has been shown to impair myocardial function, to promote arrhythmogenesis and interfere with ischaemic preconditioning in hearts isolated from healthy rats (Tosaki *et al.*, 1994). This concept has been supported by the findings that treatment with superoxide dismutase attenuates impaired function of the vascular endothelium in cholesterol-fed rabbits (Mügge *et al.*, 1991). One may therefore speculate that there might be a correlation between vascular endothelial function and ischaemic preconditioning.

Atherogenesis has been shown to induce a selective deterioration of endothelial function (for review see Flavahan, 1992). The impairment of endothelium-dependent vasodilation and the dominance of vasoconstrictor responses represent an early abnormality which may precede atherosclerosis in hypercholesterolaemic animals and humans (Tanner *et al.*, 1991; Yasue *et al.*, 1990). Vascular rings isolated from normal rabbits exposed to oxidized low density lipoproteins rapidly develop an impaired endothelial function which may be reversed by L-arginine through an increased synthesis and release of nitric oxide (NO). Drexler *et al.* (1991) have also shown that the ACh induced vasodilation of epicardial coronary arteries and coronary resistance vessels is impaired in hypercholesterolaemic human subjects with no improvement of the epicardial coronary response after intracoronary administration of L-arginine. However, they found a marked increase in coronary flow in response to ACh reflecting an amelioration of endothelial function mainly at the level of resistance vessels. NO is known to activate guanylyl cyclase resulting in an increase in cyclic guanosine 5′-monophosphate (cGMP) formation. Large conductance vessels, such as thoracic aorta and coronary arteries isolated from hypercholesterolaemic rabbits and man, have been shown to exhibit decreased cGMP formation in parallel with the impaired endothelium-dependent vasodilation (Bossaller *et al.*, 1987).

In previous studies performed in conscious rabbits, we have demonstrated that VOP-induced preconditioning is associated with an increase in cardiac cGMP concentration (Szilvassy *et al.*, 1994a), and the cardioprotective effect afforded by preconditioning is lost in rabbits made tolerant to the vasodilator effect of nitroglycerin (Szilvassy *et al.*, 1994c), a condition known to impair cGMP formation. These observations suggest that cGMP signalling may be an important trigger mechanism in ischaemic preconditioning. Consequently, a decrease in cGMP formation in the heat of hypercholesterolaemic and/or atherosclerotic animals may lead to the abolition of the preconditioning effect. The present results, showing that hypercholesterolaemia abolishes preconditioning before affecting aortic ACh-induced endothelium-dependent vasodilation, seem to be contradictory to this assumption. Clinical studies have however provided convincing evidence that the *in situ* impairment of microvascular endothelial response may be a more sensitive marker of early atherosclerotic changes than the *in vitro* impairment of the endothelial vasomotor function of large conductance vessels (Creager *et al.*, 1990; Gilligan *et al.*, 1994; Reddy *et al.*, 1994).

In conclusion, whatever the underlying mechanism of ischaemic preconditioning is, a substantial body of evidence suggests that preceding ischaemic insult triggers the formation and release of presently unidentified endogenous protective substances, the effect of which is unaltered in the healthy and normocholesterolaemic/atherosclerotic heart, notwithstanding, the release process and/or the effect of these endogenous cardioprotective substances may be abolished by hypercholesterolaemia, an important risk factor in coronary heart disease.

Acknowledgements

This work was supported by IPSEN, Paris, France, grants from OTKA F6396 and E62277, the Hungarian Space Research Office and the Hungarian Credit Bank, Budapest, Hungary. The authors thank Ilona Hatvani for her expert technical assistance.

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European Journal of Pharmacology 301 (1996) R17–R18

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Rapid communication

mpaired nitrergic relaxation of the sphincter of Oddi of hyperlipidaemic rabbits

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Received 20 February 1996; accepted 23 February 1996

bstract

Field stimulation relaxed the sphincter of Oddi muscle rings of the rabbit after incubation with phentolamine, oxprenolol and atropine ll 1 μM). The relaxation was blocked by N^G-nitro-L-arginine methyl ester (30 μM) and was reversed by 3 mM L-arginine but not arginine. Sphincter of Oddi preparations from hypercholesterolaemic rabbits exhibited contractions under the same conditions. We nclude that nitrergic relaxation is impaired in the sphincter of Oddi from hypercholesterolaemic rabbits.

ywords: Nitric oxide (NO); Hypercholesterolemia; Sphincter of Oddi

The relaxation function of the spincter of Oddi is ainly regulated by non-adrenergic non-cholinergic NANC') nerves that are essentially nitrergic in rabbits onovics et al., 1994). It is suggested that hypercholesrolaemia attenuates either the release or the effect of tric oxide (NO) in blood vessels (Verbeuren et al., 1986). evertheless, alterations in NANC relaxation of the hincter of Oddi in hypercholesterolaemia have not been vestigated. The present work aimed to study whether trergic relaxation of the sphincter of Oddi was altered in percholesterolaemic rabbits.

Groups of male New Zealand rabbits (3500–3700 g) ere fed commercial rabbit chow ($n = 6$) and chow enched with 1.5% cholesterol ($n = 6$) over 8 weeks. The rum cholesterol level was determined before and after e 8-week period as described (Szilvassy et al., 1995). ne animals were then stunned and exsanguinated. Amllary parts of sphincter of Oddi muscle rings (3 mm) ere suspended on glass hooks in an organ bath (5 ml) ntaining Krebs bicarbonate buffer (mM: NaCl 118.1, Cl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.0, $CaCl_2$ 2.5, $NaHCO_3$ 5.0, glucose 11.1) maintained at 37°C and aerated continously with 95% O_2 and 5% CO_2. The glass hooks were nnected to a force transducer for measurement of isoetric tension as described (Lonovics et al., 1994). The itial tension was set at 20 mN and the rings were allowed to equilibrate over 1 h. Then electrical field stimulation was applied as described (Lonovics et al., 1994). Contractile responses to 10 stimuli (50 V, 0.1 ms and 20 Hz) were studied. The rings were then preincubated with phentolamine, oxprenolol and atropine (all 1 μM) for 20 min. This was followed by three consecutive 20-min incubations with N^G-nitro-L-arginine methyl ester, 3 mM D-arginine or L-arginine. Field stimulation was applied after each period. Following washout, the stimulation protocol was repeated to test whether 'predrug' contractile responses could be reproduced. All compounds (Sigma, St. Louis, MO) dissolved in Krebs solution were added to the organ bath in a 50-μl volume. The data expressed as means ± S.D. were analysed with a one-way analysis of variance followed by the Bonferroni *t*-test. Changes were considered significant at $P < 0.05$.

A cholesterol-enriched diet increased serum cholesterol to 26.1 ± 4.0 vs. pre-diet 1.8 ± 0.29 mmol/l ($P < 0.001$). In rabbits fed normal chow, serum cholesterol did not change during the same period.

Field stimulation induced NANC relaxations in normal sphincters, an effect that was reversed to contractions after incubation with N^G-nitro-L-arginine methyl ester. L-Arginine but not D-arginine reversed this inhibitory effect (Fig. 1). The preparations from hypercholesterolaemic rabbits responded with contractions to field stimulation after incubation with NANC solution. Successive incubations with either arginine analogue failed to influence these contractions.

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/ S0014-2999(96)00159-8

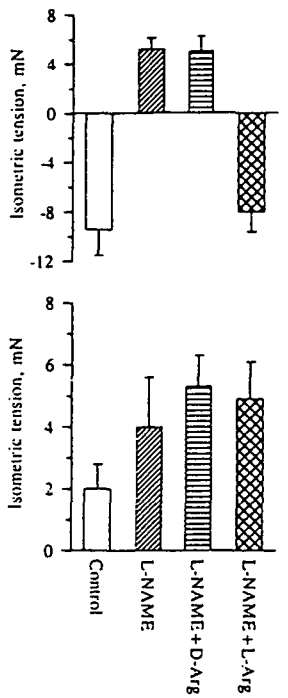

Fig. 1. Effect of electrical field stimulation on motility of isolated sphincter of Oddi from normal (upper panel) and hypercholesterolaemic (lower panel) rabbits. Maximum contraction/relaxation responses to 10 stimuli (50 V, 0.1 ms, 20 Hz) are expressed as mN (means ± S.D. obtained with 6 preparations) in the control (responses in NANC solution, i.e. after incubation with phentolamine, oxprenolol and atropine (1 μM for each) and after three consecutive incubations with 30 μM N^G-nitro-L-arginine methyl ester (L-NAME), 3 mM D-arginine and L-arginine.

These data confirm that NANC relaxation of the normal sphincter of Oddi of the rabbit is essentialy nitrergic (Lonovics et al., 1994). However, this report is the first to describe NANC relaxation of this sphincter as impaired by hypercholesterolaemia. In the vasculature, functional defects have been identified in endothelial cells in hypercholesterolaemia (Verbeuren et al., 1986). We also have found that 8-week exposure to a 1.5% cholesterol-enriched diet results in impairment of endothelium-dependent vasodilation (Szilvassy et al., 1995). Previously, reduced endothelium-dependent vasorelaxation due to hypercholesterolaemia has been proposed to have as basis a reduced formation and/or release of endothelium-derived relaxing factor (EDRF) identified as NO. Nevertheless, the diminished nitrergic response may result from more rapid inactivation of the NO released. Ohara et al. (1993) have shown an increased superoxide anion production in hypercholesterolaemia and an improvement of EDRF-dependent relaxation by superoxide dismutase; however, inactivation of NO by low-density lipoproteins may also be involved (Jacobs et al., 1990). It is also possible that NO is less active on gastrointestinal smooth muscle cells because of alterations in the signal transduction pathway within the muscle cells. Studies with exogenous NO donors on sphincter of Oddi motility would clarify this point since, if the latter were true, relaxation responses to nitroglycerin or sodium nitroprusside should also be disturbed in hypercholesterolaemia.

In summary, the study demonstrated the impairment of the endogenous relaxation mechanism of the sphinter of Oddi from hypercholesterolaemic animals, indicating a possible role of hypercholesterolaemia in papillary dysfunction-related diseases such as sphincter of Oddi dyskinesia and/or acute pancreatitis.

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Reprinted from

European Journal of Pharmacology

European Journal of Pharmacology 255 (1994) 117–122

Regional differences in nitric oxide-mediated relaxation of the rabbit sphincter of Oddi

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(Received 22 November 1993; revised MS received 29 December 1993; accepted 4 January 1994)

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European Journal of Pharmacology 255 (1994) 117–122

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Regional differences in nitric oxide-mediated relaxation of the rabbit sphincter of Oddi

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(Received 22 November 1993; revised MS received 29 December 1993; accepted 4 January 1994)

Abstract

We studied the role of the L-arginine–nitric oxide (NO) pathway in non-adrenergic, non-cholinergic (NANC) relaxation of the rabbit sphincter of Oddi by recording changes in isometric tension in response to electrical field stimulation in two series of experiments. In a first set of experiments, biliary sphincters of Oddi removed from New Zealand white rabbits were placed horizontally in an organ bath containing oxygenized, buffered (pH 7.4) Krebs solution. Contractile responses of the whole sphincter to field stimulation were determined. In the second set of experiments, sphincter of Oddi was divided into two parts and the effects of field stimulation were studied separately on areas close to the duodenal papilla (area I) and areas close to the common bile duct (area II). In the whole sphincter of Oddi, field stimulation induced an initial twitch-like contraction followed by relaxation proportional to the number of stimuli (3 and 10 stimuli at 20 Hz, 50 V, 0.1 ms). The magnitude of the contractile responses was considerably reduced by 1 μM atropine, phentolamine and oxprenolol (NANC solution). Field stimulation produced dose-dependent contractions of both segments of sphincter of Oddi in response to the same protocol as used with whole sphincter of Oddi. However, preincubation with NANC solution produced monophasic relaxations in response to field stimulation in area I, whereas area II preparations such as the whole sphincter of Oddi responded with contractions followed by minimal relaxations. Field stimulation failed to induce either contractions or relaxations in the presence of 1 μM tetrodotoxin. 30 μM N^G-nitro-L-arginine methyl ester abolished NANC relaxation in area I, but did not influence significantly contractile responses of area II, or the whole sphincter of Oddi. L-Arginine but not D-arginine (both 3 mM) reversed the inhibitory effect of N^G-nitro-L-arginine on NANC relaxation in area I. We conclude that (i) the L-arginine–NO pathway is involved in NANC relaxation of sphincter of Oddi segment close to the duodenal papilla and (ii) that there are regional differences in NO-mediated relaxation of the sphincter of Oddi in rabbits.

Key words: Nitric oxide (NO); Sphincter of Oddi; Field stimulation; Neural relaxation

1. Introduction

Nitric oxide (NO) formed from L-arginine by a constitutive NO synthase in endothelial cells (Moncada et al., 1991) was shown to play an important role in regulation of gastrointestinal blood flow (Pique at al., 1989; Pizcueta et al., 1992). From studies in vitro, NO has also been proposed as a mediator of non-adrenergic, non-cholinergic (NANC) relaxation of the guinea pig stomach and ileum (Desai et al., 1991; Gustafsson et al., 1990), of the rat stomach, colon, duodenum and ileum (Li and Rand, 1990; Hata et al., 1990; Irie et al., 1991; Kanada et al., 1992), of the canine ileo-cecal junction, ileum, duodenum and colon (Boeckxstaens et al., 1990; Toda et al., 1990; Dalziel et al., 1991) and of human colon and internal anal sphincter (Burleigh, 1992). Pauletzki et al. (1993) have shown that in the sphincter of Oddi, L-arginine–NO pathways may play a major role in neural relaxation in the guinea pig. Nevertheless, regional differences in the excitatory and inhibitory innervations of the sphincter of Oddi of the latter species have also been well documented (Hirose and Ito, 1991). Therefore, the present study was concerned with the question as to whether there are regional differences in the involvement of the L-arginine–NO pathway in NANC relaxation of circular muscle of the rabbit sphincter of Oddi in vitro.

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SSDI 0014-2999(94)00042-6

2. Materials and methods

Adult male New Zealand white rabbits weighing 2500–3000 g were stunned and exsanguinated. The duodenum with the sphincter of Oddi and the common bile duct were removed. Sphincter of Oddi muscle rings of approximately 4 mm length were prepared, cleaned of fat, adhering connective tissue and the underlying duodenum. Following removal of papilla Vateri, the muscle rings were mounted horizontally on two small L-shaped glass hooks one of which was connected to a force transducer (SG-O2, Experimetria, Budapest, Hungary) attached to a six-channel polygraph (R61 6CH, Mikromed, Budapest, Hungary) for measurement and recording of isometric tension. The experiments were carried out in an organ bath (5 ml) containing Krebs bicarbonate buffer (mM: NaCl 118.1, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.0, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1) which was maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2. The pH of the solution was kept constant at 7.4 ± 0.05. The initial tension was set at 10 milliNewton (mN) and the sphincter of Oddi preparations were allowed to equilibrate for 60 min before the experiments were started, during which period the sphincters developed characteristic 14–19 per min rhythmic contractions (Fig. 2). Muscle rings with mechanical quiescence were excluded from the experiments.

Electrical field stimulation with 50 V square impulses of 0.1 ms duration was applied via two platinum wire electrodes positioned at each side of the muscle rings connected to an Experimetria ST 02 (Budapest, Hungary) two-channel programmable stimulator.

2.1. Experimental protocol

In a first series of experiments, contractile responses of the muscle rings to two consecutive trains of impulses consisting of 3 and 10 stimuli (50 V, 0.1 ms and 20 Hz) divided by a 2-min interval, were studied. The muscle rings were then preincubated with phentolamine, oxprenolol and atropine (all 1 μM) for 30 min and the field stimulation protocol was repeated. These drugs were added to block adrenoceptors and muscarinic receptors (NANC solution). Then N^G-nitro-L-arginine methyl ester, an inhibitor of NO synthase (Rees et al., 1990), at a concentration of 30 μM was added to the solution. The sphincters were subjected to the same field stimulation protocol after a 30-min incubation with N^G-nitro-L-arginine methyl ester. These procedures were followed by two successive incubations with 3 mM D-arginine and 3 mM L-arginine (30 min). The field stimulation protocol was repeated after both D- and L-arginine. Following washout, the field stimulation protocol was repeated to test whether predrug contractile responses could be reproduced. The muscle rings with altered spontaneous activity or exhibiting any significant increase in contractile responses to field (compared to control stimulations) were n further experiments. In the second series ments, the same muscle rings were separate equal regions: area close to the papilla Vat and area close to the common bile duct (a following an additional 30-min incubation, mental protocol described for the whole sp run again. In a third series of experiments rate sphincter preparations, field stimulatio formed after a 30-min preincubation w tetrodotoxin.

After completion of the experiments, th of Oddi muscle rings were examined histo an unbiased histologist who confirmed that ter ring specimens were uncontaminated b duodenal tissue.

2.2. Drugs and chemicals

N^G-Nitro-L-arginine methyl ester, L-argi chloride, D-arginine hydrochloride and t were obtained from Sigma Chemical Co Louis, MO, USA). Phentolamine mesylate, hydrochloride and atropine sulphate were from EGIS Chemicals (Budapest, Hungary) pounds were dissolved in Krebs solution directly to the organ bath in a 50 μl volum

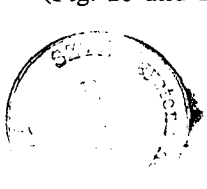
2.3. Statistical analysis

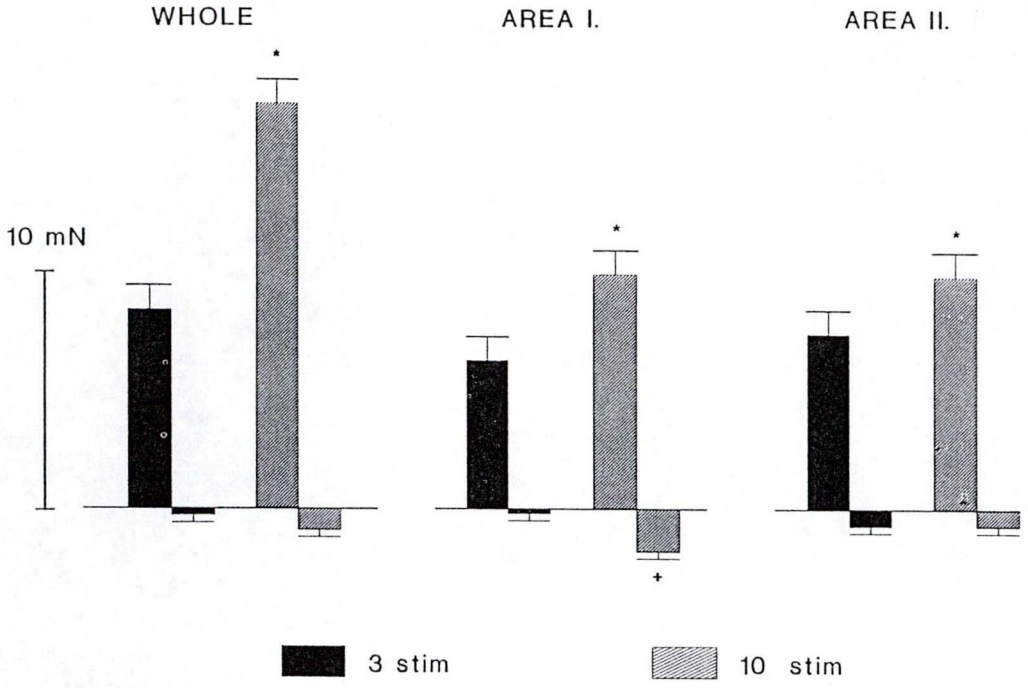
Results indicated as peak contraction (mN) in response to field stimulation are s means ± S.E.M. of 6 experiments. The dif tween groups was evaluated by Student' paired data, and $P < 0.05$ was taken as sig

3. Results

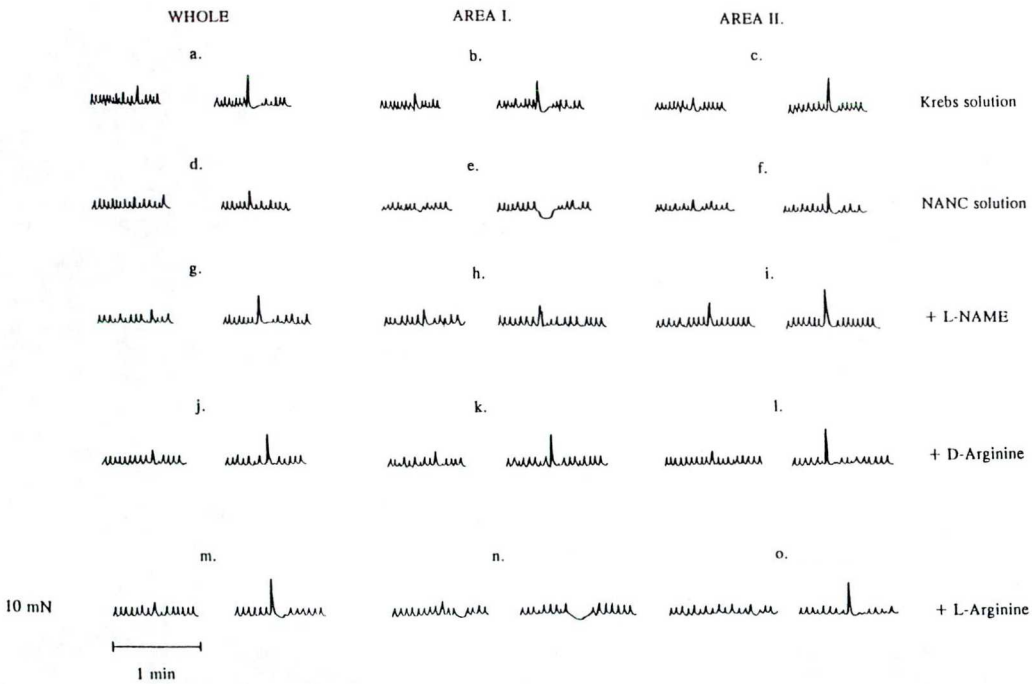
Repetitive field stimulation evoked twitch-like contraction followed by relaxa whole sphincter of Oddi, in areas I and II and Fig. 1). The magnitude of contraction ations was proportional to the number of s

Combined application of 1 μM atropine and phentolamine markedly reduced the a field stimulation-induced initial twitch-li tions in the whole sphincter and area II (F Fig. 3a), and, completely abolished contrac I (Fig. 2e and Fig. 3a). Electrical filed resulted in monophasic relaxations of an portional to the number of stimuli in thi (Fig. 2e and Fig. 3a). In the presence an





Effect of electrical field stimulation on isolated rabbit sphincter of Oddi (SO) motility. Maximum contraction/relaxation in response to mulation (3 and 10 stimuli at 50 V, 20 Hz, 0.1 ms) is expressed in milliNewton (mN). Area I represents the sphincter area close to the al papilla, whereas area II indicates sphincter of Oddi regions close to the common bile duct. Positive values indicate contractions; e values denote relaxations (see Materials and methods). Data are means ± standard error of the mean (S.E.M.) obtained in 6 ients. * Significant difference between maximum contractions obtained with 3 and 10 stimuli at $P < 0.05$. + Significant difference n maximum amplitude of relaxations provoked by 3 and 10 stimuli at $P < 0.05$.



Original tracings indicating the effect of field stimulation on motility of isolated rabbit sphincter of Oddi. For explanation of 'Whole', I' or 'Area II', see Materials and methods and Fig. 1 legend. Left tracings of each plot (a, b, c, etc.) represent changes in isometric tension ponse to 3 stimuli; right tracings represent tension changes produced by 10 stimuli (50 V, 20 Hz, 0.1 ms). First row (a, b and c) indicates obtained in sphincter of Oddi preparations incubated in normal Krebs solution. Second row (d, e and f) indicates results in NANC on. Third row (g, h and i) shows the effect of L-NAME (N^G-nitro-L-arginine methyl ester, 30 μM) on NANC responses to field stimulation. n row (j, k and l) represents findings after additional incubation with D-arginine (3 mM). Fifth row (m, n and o) indicates the effect of onal L-arginine (3 mM).

these agents, responses elicited by electrical field stimulation were completely blocked by tetrodotoxin (1 μM) and were therefore regarded as nerve responses (data not shown). Nevertheless, additional incubation with L-NAME (30 μM) reversed field stimulation-induced relaxation of area I in NANC solution (Fig. 2h and Fig. 3b), whereas a complete recovery of the magnitude of stimulation-induced initial twitch-like contractions was seen in studies with the whole sphincter of Oddi and area II muscle rings (Fig. 2g,i and Fig. 3b). L-Arginine but not D-arginine (both 3 mM) was found to antagonize the inhibitory effect of N^G-nitro-L-arginine methyl ester on field stimulation-induced NANC relaxation in the sphincter area I (Fig. 2k,n and Fig. 3c,d).

4. Discussion

These results indicate that field stimulation according to a standardized stimulation protocol results in different mechanical responses in different regions isolated sphincter of Oddi muscle rings of the rabb preincubated with NANC solution (phentolamine, c prenolol and atropine combination). Since the sphincter of Oddi preparations did not include t papilla Vateri (see Materials and methods), area mainly represents the ampulla of the sphincter of Od whereas area II can be regarded as the choledoch sphincter. Our results suggest that field stimulati produces NANC relaxation in area I but we failed reproduce the characteristic phenomenon of NAN relaxation in studies with the whole sphincter and ar II muscle rings. N^G-Nitro-L-arginine methyl ester, inhibitor of NO synthase (Rees et al., 1990) was fou to completely abolish NANC relaxation which could antagonized by L-arginine but not by D-argini Therefore we think that the results provide eviden for the involvement of the L-arginine–NO pathway NANC relaxation of the ampullary part of the rab sphincter of Oddi.

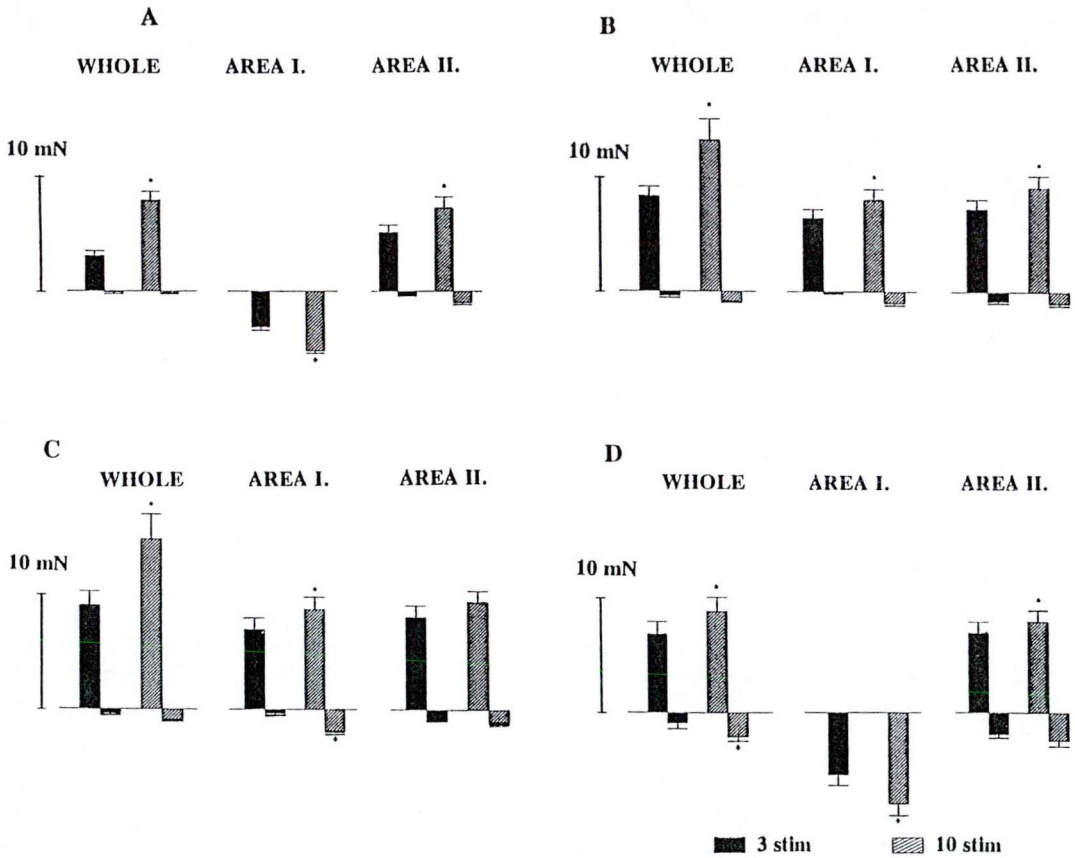


Fig. 3. Possible involvement of the L-arginine–NO pathway in NANC relaxation in the ampullary part of the rabbit sphincter of Oddi. (A) E of combined preincubation with phentolamine, oxprenolol and atropine (all 1 μM) on field stimulation-induced changes in isometric tensio the rabbit sphincter of Oddi. For details see Materials and methods and Fig. 1 legend. (B) Effect of N^G-nitro-L-arginine methyl ester (30 μM NANC mechanical responses to field stimulation in isolated rabbit sphincter of Oddi. (C) Incubation with D-arginine (3 mM) did not influ the inhibitory effect of N^G-nitro-L-arginine methyl ester (30 μM) on NANC relaxation of the rabbit sphincter of Oddi. (D) Complete recove L-arginine (3 mM) of NANC relaxation of isolated rabbit sphincter of Oddi antagonized by N^G-nitro-L-arginine methyl ester (30 μM). Dat expressed as means $\pm$ S.E.M. obtained in 6 experiments. * Significant difference between maximum contractions obtained with 3 and 10 st at $P < 0.05$. $^+$ Significant difference between maximum amplitude of relaxations provoked by 3 and 10 stimuli at $P < 0.05$.

The intrinsic innervation of the choledochoduodenal unction of mammals has received relatively little attention and has been predominantly studied by recording contractile activity of muscle strips in response to electrical nerve stimulation. The literature provides only ncomplete descriptions of the sphincter of Oddi innervation in felines (Dahlstrand et al., 1989a,b), in guinea igs (Allen et al., 1984; Goehler et al., 1988), in dogs Sievert et al., 1988) and in the opossum (Parodi et al., 990). Sand et al. (1993) first described the localisation f vasoactive intestinal peptide, bombesin, neuropeptide Y, peptide histidine-isoleucine, calcitonin gene-related peptide, galanin, substance P and serotonin in he pig sphincter of Oddi, bile duct, gallbladder and uodenum. According to our present knowledge no orphological studies have been conducted to characterize the innervation of the rabbit biliary tract regarding neurotransmitters and synaptic modulators. However, as the choledochoduodenal junction is densely nervated (Cai and Gabella, 1983), neural control echanisms are believed to play an important role in ie regulation of sphincter of Oddi motility. Intrinsic holinergic nerves were found to mediate contractions f the isolated rabbit sphincter of Oddi (Azuma and ujiwara, 1973) and of the terminal bile duct of the uinea pig (Kudoh et al., 1981). As NO released from ie non-adrenergic non-cholinergic component of nteric nervous system has been shown to play a critial role in the coordinated propagation of gut contents, articularly in sphincter relaxation (Ward et al., 1992; sthaus and Galligan, 1992), it is possible that the arginine–NO pathway might be involved in NANC laxation of the sphincter of Oddi as well. Pauletzki et . (1993) provided evidence for the involvement of O-mediated NANC relaxation in the guinea pig hincter of Oddi. Our findings seem to support the ncept of NO-mediated neural relaxation in the rabt sphincter of Oddi. On the other hand, the cholechal sphincter failed to show any NANC relaxation der our experimental conditions.

Electrical field stimulation is commonly used as a nvenient method to excite neurons in various kinds tissue preparations (Kanada et al., 1992), and was ed in the present study to induce NANC inhibitory sponse in sphincter of Oddi muscle rings. Nevertheless, it is not easy to interpret data obtained by this ethod as it stimulates all kinds of neurons in the eparation and only the overall response induced by ese various neurons can be observed. Therefore the ct, that N^G-nitro-L-arginine methyl ester can reverse ANC relaxation to a contraction as a particular reonse of area I sphincter of Oddi preparations to field mulation, may reflect the dominance of an N^G-tro-L-arginine methyl ester sensitive relaxation mechism in the ampullary region of the rabbit sphincter Oddi. Notwithstanding, one would expect that a NO synthase inhibitor such as N^G-nitro-L-arginine methyl ester could antagonize NO-mediated smooth muscle relaxation selectively without affecting the contractile component of responses to field stimulation. The present results, however, reveal that an additional incubation with N^G-nitro-L-arginine methyl ester added to NANC solution results in contractions in response to field stimulation instead of a simple antagonism of field stimulation-induced relaxation in the ampullary region. We think that this latter finding can at least in part be explained by a neuromodulatory effect of NO being able to attenuate the effect of excitatory neurotransmitters in this region (Moncada, 1992) or by a partial interaction between N^G-nitro-L-arginine methyl ester and muscarinic receptors, i.e. the effect of atropine (Buxton et al., 1993). On the other hand, additional incubation with N^G-nitro-L-arginine methyl ester did not result in different NANC contractions in area I and area II muscle rings.

VIP has also been designated as another candidate as NANC neurotransmitter relaxing the sphincter of Oddi (Dahlstrand et al., 1989a,b). Nevertheless, VIP-induced smooth muscle relaxation in the internal anal sphincter (Rattan and Chadker, 1992) and rat colon (Grider, 1993) was found at least in part mediated by NO. It was also concluded that, during neurally induced relaxation, NO was produced in the smooth muscle cells and neurons by the action of VIP, and NO was found to enhance VIP release from neurons in response to field stimulation. By reason of these interactions between VIP and NO-mediated processes, it could be speculated that VIP might serve as a transmitter to trigger NO release with a significant attenuation of VIP-induced relaxation in the presence of a NO synthase inhibitor.

Whatever the precise mechanism is, we conclude that the L-arginine–NO pathway is of dominant influence on NANC relaxation of sphincter of Oddi segments close to the duodenal papilla and the results suggest that there are regional differences in the involvement of L-arginine–NO pathway into the mechanisms of neural relaxation of the rabbit sphincter of Oddi. We think that, apart from their theoretical interest, these findings might be of clinical utility because identification of mediators and mechanisms of NANC relaxation in different parts of the gastrointestinal tract might support efforts to find the appropriate therapeutic strategy to confer protection on patients at risk of gastrointestinal motility disorders.

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J. Pharmacol. (1994), 112, 999–1001

SPECIAL REPORT

oss of preconditioning in rabbits with vascular tolerance to itroglycerin

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A preceding right ventricular overdrive pacing (VOP) of 500 b.p.m. for 5 min, markedly reduced the severity of global myocardial ischaemia produced by a subsequent 5-min VOP in conscious rabbits. This VOP-induced preconditioning developed in parallel with an increase in cardiac cyclic guanosine 3':5'-monophosphate (cyclic GMP) content. VOP-induced preconditioning was abolished when the animals had been made tolerant to the vasodilator effect of nitroglycerin (NG). In the heart of the NG-tolerant rabbits, neither VOP nor preconditioning increased cyclic GMP content. This suggests that changes by NG tolerance of cyclic GMP metabolism may account for the loss of VOP-induced preconditioning.

ywords: Ischaemic preconditioning; nitroglycerin tolerance; cyclic GMP; ST-segment elevation; LVEDP; rabbit heart

roduction The intracellular messenger, cyclic guanosine 5'-monophosphate (cyclic GMP) has been suggested to y a crucial role in the mechanism of the anti-ischaemic ict of preconditioning induced by ventricular overdrive ing (VOP) in conscious rabbits (Szilvassy *et al.*, 1994). ults obtained with anaesthetized dogs (Vegh *et al.*, 1992) suggest that nitric oxide (NO), a stimulator of soluble nylate cyclase, contributes to the antiarrhythmic effect of conditioning produced by brief coronary artery occlusions. reover, inhibition of the L-arginine-NO pathway has been nd to abolish the antiarrhythmic effect of bradykinin, a ative mediator of preconditioning (Vegh *et al.*, 1993). eral studies have shown that the tolerance to organic oesters is accompanied by reduced production and in-ased breakdown of cyclic GMP in vascular and non-cular tissues (Axelsson & Ahlner, 1987). Therefore, we mined whether nitroglycerin (NG) tolerance influenced P-induced preconditioning in conscious rabbits.

thods Male New Zealand white rabbits (2500–3000 g) e anaesthetized with 15 mg kg^{-1} diazepam (Sigma, St is, MO, U.S.A.) and 10 mg kg^{-1} ketamine (EGIS, lapest, Hungary) given intravenously. A bipolar 'French lectrode catheter was introduced into the apex of the right tricle for pacing and recording the intracavital electro-m (Szilvassy *et al.*, 1993a,b). Polyethylene catheters were rted into the left ventricular cavity and the central ear ry to measure intraventricular pressure and mean arterial od pressure (MABP), respectively. One week after gery, the rabbits were given 150 μl kg^{-1} ethanol, the sol-t for NG, subcutaneously four times a day for three days. t morning global myocardial ischaemia was provoked by P of 500 b.p.m. for 5 min. Average intracavital ST-nent elevation during the first five cardiac cycles at sinus hm after VOP produced the data for evaluation. Left ricular end-diastolic pressure (LVEDP) was measured re and immediately after VOP. This VOP was then given 5 min followed by a second 5-min VOP, and the para-ers were determined again. The first VOP served to nate electrophysiological and haemodynamic changes due lobal myocardial ischaemia and to induce precondition-The second VOP was to test the preconditioning effect the parameters measured. The rabbits were then given g kg^{-1} NG (EGIS, Budapest, Hungary) subcutaneously times a day for three days to induce NG tolerance and the pacing protocol was repeated. The effect of an intravenous bolus of 30 μg kg^{-1} NG on MABP was tested before and after the three-day NG administration. In the second set of experiments, two groups of electrode catheter-instrumented rabbits (15 of each) underwent the three-day treatment with NG or solvent, respectively. Anaesthetized, open-chest, artificially ventilated rabbits were subjected to VOP of 500 b.p.m. for 5 min as described (Szilvassy *et al.*, 1993a). Normal and NG-tolerant rabbits were divided into three subgroups. One was subjected to a single VOP, then immediately after VOP, samples were taken from the apex of the left ventricle for cyclic GMP determination. Another five rabbits underwent two periods of VOP with an interpacing period of 5 min, and samples were taken immediately after the second VOP. A group was killed for measuring baseline cyclic GMP values. Cardiac cyclic GMP content was measured by means of radioimmunoassay using Amersham kits as described (Szilvassy *et al.*, 1993a). Data expressed as means ± s.d. were statistically analyzed by ANOVA followed by a modified *t* test for multiple comparisons according to Bonferroni.

Results In non tolerant rabbits, 30 μg kg^{-1} intravenous NG decreased MABP from 88 ± 5.8 mmHg to 72 ± 5.1 mmHg ($P < 0.01$), whereas it did not change MABP in tolerant animals (91 ± 4.4 mmHg vs 93 ± 5.2 mmHg). A single VOP induced an intracavital ST-segment elevation of 1.1 ± 0.16 mV in non tolerant rabbits (Figure 1a); nevertheless, when this was preceded by a preconditioning VOP, the ST-segment elevation decreased to 0.6 ± 0.07 mV ($P < 0.001$). In tolerant rabbits, ST-segment elevation induced by a single VOP was more marked than in controls (1.5 ± 0.22 mV vs 1.1 ± 0.16 mV, $P < 0.05$), and it remained unchanged after a preconditioning VOP. The increase in LVEDP produced by a single VOP was markedly decreased by a preconditioning VOP in non tolerant but not in NG tolerant rabbits (Figure 1b). In non tolerant animals, a single VOP significantly increased cardiac cyclic GMP content as compared to resting values (Figure 1c) and preconditioning slightly amplified the VOP-induced cyclic GMP increase. In tolerant animals, however, neither a single VOP nor consecutive periods of VOP changed cardiac cyclic GMP content. Resting LVEDP and cardiac cyclic GMP of NG tolerant rabbits did not significantly differ from that of non tolerant ones (data not shown).

Discussion These results confirm previous findings that the global myocardial ischaemia induced by VOP is markedly

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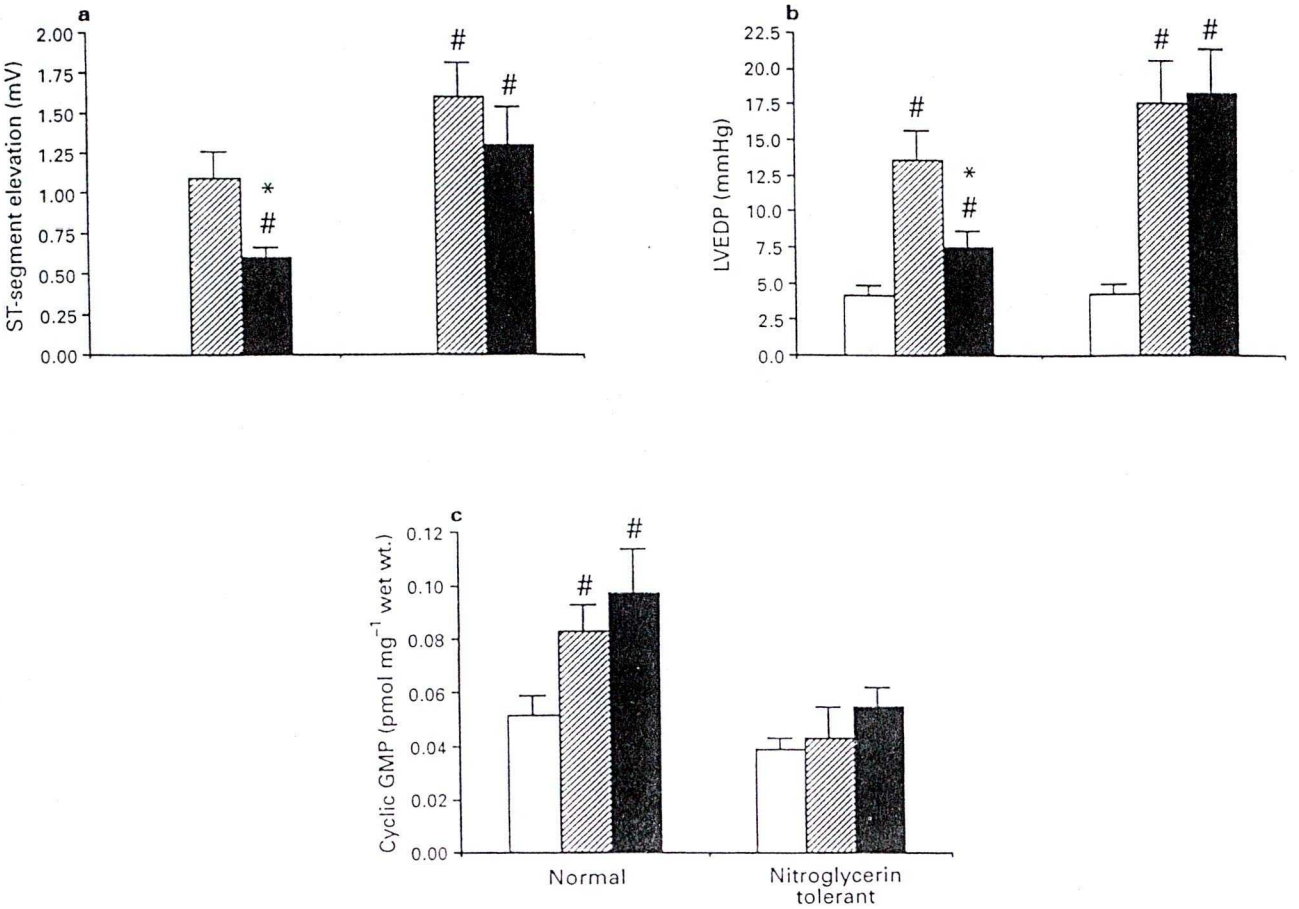

Figure 1 Effect of ventricular overdrive pacing (VOP) on (a) intracavital ST-segment elevation and (b) left ventricular end-diastolic pressure (LVEDP) in conscious rabbits ($n = 6$) and (c) on cardiac cyclic GMP content in anaesthetized rabbits ($n = 5$). Open columns show resting, hatched columns non preconditioned values measured after a single VOP (500 b.p.m. for 5 min), solid columns designate values measured after a 5 min VOP preceded by a preconditioning VOP of the same rate and duration with an interpacing period of 5 min. 'Normal' values are obtained from solvent-treated animals. 'Nitroglycerin tolerant' values derived from rabbits treated with 50 mg kg^{-1} nitroglycerin (NG) four times a day for three days. Data are means ± s.d.; #significant difference between resting and postpacing; *between non preconditioned and preconditioned values, $P < 0.05$.

attenuated by a preceding ischaemic challenge provoked by VOP (Szilvassy *et al.*, 1994). The results also indicate that this protection is lost when tolerance to the vasodilator effect of NG develops. Ischaemic preconditioning is believed to be due to the increased formation and release of endogenous cardioprotective substances. These substances may derive from the vascular endothelium or cardiac myocytes and stimulate soluble guanylate cyclase either directly like NO (Moncada *et al.*, 1991), or indirectly like bradykinin via releasing NO (Vegh *et al.*, 1992; 1993). An increased formation of cyclic GMP may therefore be considered an important step in the signal transduction pathways involved in preconditioning. This concept is in accord with our previous finding that an increase in cardiac cyclic GMP content correlates with the time course of the anti-ischaemic effect of VOP-induced preconditioning (Szilvassy *et al.*, 1994). Pharmacological inhibition of cyclic GMP breakdown has also been found to have an anti-ischaemic effect in conscious rabbits (Szilvassy *et al.*, 1993b). The present data, showing that VOP-induced preconditioning is lost in NG tolerance in association with the inability of VOP to increase myocardial cyclic GMP content, suggest that cyclic GMP signalling contributes to the protection of the ischaemic myocardium.

The mechanism of NG tolerance is unclear, although different hypotheses have been advanced, suggesting (i) the importance of pharmacokinetic factors, (ii) the oxidation of critical sulphydryl groups in the receptor inducing a low affinity state (Axelsson & Ahlner, 1987), and (iii) alterations in the formation and breakdown of cyclic GMP. Cyclic GMP is an important mediator of vasorelaxation induced by NG. Keith *et al.* (1982) found an inhibition of NG-induced cyclic GMP generation in aortic strips of NG-tolerant rats; however, the relaxation induced by 8-bromo-cyclic GMP, a stable, membrane-penetrating cyclic GMP analogue, was unaltered, indicating that, once it is formed, the intracellular action of cyclic GMP is unimpaired. In NG tolerance, Axelsson & Andersson (1983) have shown that guanylate cyclase activity is decreased, while cyclic GMP hydrolysis is enhanced. These findings might explain the impairment of preconditioning in NG-tolerant rabbits. Besides theoretical considerations, our results emphasize the potential risk of nitrate tolerance in clinical patients maintained on prolonged nitrate therapy.

This work was supported by a grant from Hungarian National Health Foundation (OTKA) 6396, Budapest, Hungary.

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(Received April 13, 1994
Accepted April 29, 1994)

Mechanism of vasodilation by cohlear nerve stimulation. Role of calcitonine gene-related peptide.

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Summary

Rabbit aortic rings pre-contracted with 1 μM phenylephrine were exposed to organ fluid of isolated guinea pig cochleas which had been subjected to electrical field stimulation (FS, 50 Hz, 50 V, 0.2 ms over 2 minutes). This resulted in an endothelium-dependent relaxation of the vessel rings sensitive to glibenclamide, an ATP-sensitive K^+ channel blocker. Tetrodotoxin (1 μM) added to the cochlear fluid blocked the vasorelaxant effect of cochlear FS and it attenuated vasorelaxation when added to aortic rings. The relaxation response parallelled an increase in level of calcitonin gene related peptide (CGRP) in both cochlear and vascular organ fluids from undetectable pre-stimulation values to 0.12 ± 0.029 and 0.44 ± 0.051 nM, respectively. We conclude that CGRP possibly contributes to cochlear nerve stimulation-induced endothelium-dependent vasorelaxation.

Key words: cochlear stimulation, CGRP, glibenclamide, neural vasorelaxation.

INTRODUCTION

Calcitonin gene-related peptide (CGRP) and nitric oxide (NO) have been found to play a modulatory role in cochlear function [1-3]. Besides their neuromodulatory role, both neurotransmitters are known to induce vasodilation [4-7]. We have found that electrical activation of the cochlea of the guinea pig produces an endothelium-dependent vasorelaxation with involvement of a glibenclamide-sensitive mechanism [8]. This mechanism has been found to be underlain by an increased endothelial production of NO with an increase in vascular concentration of cyclic AMP and cyclic GMP. Both NO acting through cyclic GMP [5] and CGRP acting through cyclic AMP [4] have been found to activate ATP-sensitive potassium (K_{ATP}) channels in vascular tissue [4,6,7,9]. Moreover, these neurotransmitters contribute together to non-adrenergic, non-cholinergic (NANC) vasorelaxation with the involvement of endothelium-dependent mechanisms [6,7,10]. The present work was therefore to provide evidence for the contribution of CGRP to K_{ATP} channel-dependent vasorelaxation deriving from electrical activation of the isolated cochlea of the guinea pig by direct determination of CGRP.

METHODS

These experiments conform to NIH guidelines for the care and use of laboratory animals. Cochleas were prepared from pentobarbitone- (40 mg kg^{-1})-anaesthetized healthy adult guinea pigs and placed into an organ chamber (5 ml) containing oxygenized Krebs bicarbonate buffer. To release neurotransmitters from the cochlea, electrical field stimulation (FS) with 50 V square impulses of 0.1 ms duration at a rate of 50 Hz was applied over 2 minutes by means of an EXPERIMETRIA (London, England) programmable stimulator as described [8]. To study the vascular effects of neurotransmitters released from the cochleas, 4 mm long aortic rings were prepared from adult male New Zealand white rabbits weighing 3000-3500 g and tested for changes in isometric tension as described [8,11]. The vessel rings were incubated in separate organ chambers filled with Krebs bicarbonate buffer (5 ml) connected to that of the cochleas. The experiments were carried out with aortic rings having functionally intact endothelium and with those from which the endothelium had been removed as described [11]. Functional integrity of vascular endothelium was confirmed by exposure of the rings to 1 μM acetylcholine chloride as described [11]. Rings exhibiting a significant relaxation response were referred to as rings with intact endothelium, whereas preparations with no relaxation or showing contractions were noticed as 'endothelium free' preparations. The aortic rings were pre-contracted with 1 μM phenylephrine and after a stable contraction was obtained, the rings were exposed to 1 ml of the organ fluid of the cochleas in which the FS had been accomplished ('cochlear

fluid'). Subsequently, the rings were exposed to the "cochlear fluid" in the presence of cumulative increases in glibenclamide concentration at log concentration increments. The FS protocol was applied 5 minutes following administration of each glibenclamide concentration. Finally, after washout, the FS protocol was repeated in the presence of 1 μM tetrodotoxin (TTX) added to either the cochleas or the vessel rings. Phenylephrine, glibenclamide, acetylcholine chloride and TTX were obtained from Sigma (St. Louis, Mo, USA). Stock solution for TTX and phenylephrine were prepared in Krebs solution whereas glibenclamide was dissolved in dimethylsulfoxide. The compounds were added directly to the organ bath in 30 μl volume.

Pre-and post-stimulation CGRP contents in organ fluid of the cochleas or the vessel rings were determined by means of radioimmunoassay using Peninsula (Belmont CA) kits as described [12].

The data expressed as means $\pm$ S.D. were statistically analyzed by analysis of variance followed by Bonferroni's *t*- test [13]. The level of significance was $p < 0.05$ at $n = 6$ in each group.

RESULTS

The aortic rings relaxed in response to exposure to fluid of the stimulated cochleas. The relaxation response was blocked by removal of the endothelial layer (Fig.1). TTX added to the cochlear fluid also blocked the effect of FS and it attenuated vasorelaxation when added to aortic rings (Fig.1). Glibenclamide attenuated relaxation produced by the cochlear fluid (Fig.2). The solvent for glibenclamide (dimethyl-sulfoxide) was without effect (data not shown).

FS increased CGRP concentration from undetectable pre-stimulation values to 0.12 ± 0.029 vs 0.44 ± 0.051 nM ($p < 0.001$) in organ fluids of the cochleas vs in that of the vessel rings. TTX given to either the cochleas or the rings, blocked CGRP-increase in the medium of vascular preparations.

DISCUSSION

The present results confirm our previous finding that electrical FS of isolated guinea pig cochleas produces a partially glibenclamide-sensitive relaxation in rabbit aortic rings, which is endothelium-dependent [8]. In parallel with the relaxation response, a significant increase in CGRP concentration was seen in organ fluid of both the cochlear preparations and the vessel rings. Nevertheless, CGRP concentration of the vascular fluid much exceeded that of the cochlear one indicating the presence of a currently undefined 'amplifying' mechanism in the

aortic tissue, which also requires the presence of the endothelial layer. We have previously found the endothelium-dependent vasorelaxation to be blocked by inhibition of NO synthesis indicating the relaxation response to be essentially nitric oxide-mediated in nature [8]. Since TTX added to the cochlear preparations blocked the vascular response, we confirmed that substance(s) from the electrically stimulated cochlea producing vasodilation are of neural origin [8].

CGRP has long been recognized to play a neuromodulatory role in both cochlear function [3] and NANC regulation of vascular tone [4,6,7]. Regarding its vascular effects, CGRP has been shown to mainly utilize K_{ATP} channel activation to produce vasodilation [4]. Our present finding that pharmacologically relevant concentrations of CGRP are produced in the vessel wall in response to cochlear stimulation would have provided an explanation for the glibenclamide-sensitive vasorelaxation. Nevertheless, the relaxant effect of CGRP is considered mainly endothelium-independent [4], whereas cochlear stimulation-induced vasodilation is completely blocked by removal of the vascular endothelium. Nevertheless, a very recent report by Kakuyama et al [7] suggests that endothelial NO is involved in vasorelaxation produced by sensory neuropeptides. Moreover, the ability of the vessel rings to produce increased amounts of CGRP at least under our experimental conditions, also requires the presence of the endothelial layer. However, the lack of complete inhibition of cochlear stimulation-induced vasorelaxation by glibenclamide suggests either an incomplete blockade of these channels or the presence of an additional, K_{ATP} channel-independent relaxation mechanism possibly related to NO. Based on the present results, it is suggested that endothelial NO somehow may interact with NANC neural elements to release CGRP from nerve terminals, an effect to enhance

or contribute to its 'per se' relaxant effect.

The results support the concept that the endothelial layer is a transducer of important vascular signals to which the vessels respond to ensure adequate tissue perfusion. These results also imply the potential use of K_{ATP} channel openers to support the clinical use of cochlear electrical stimulation.

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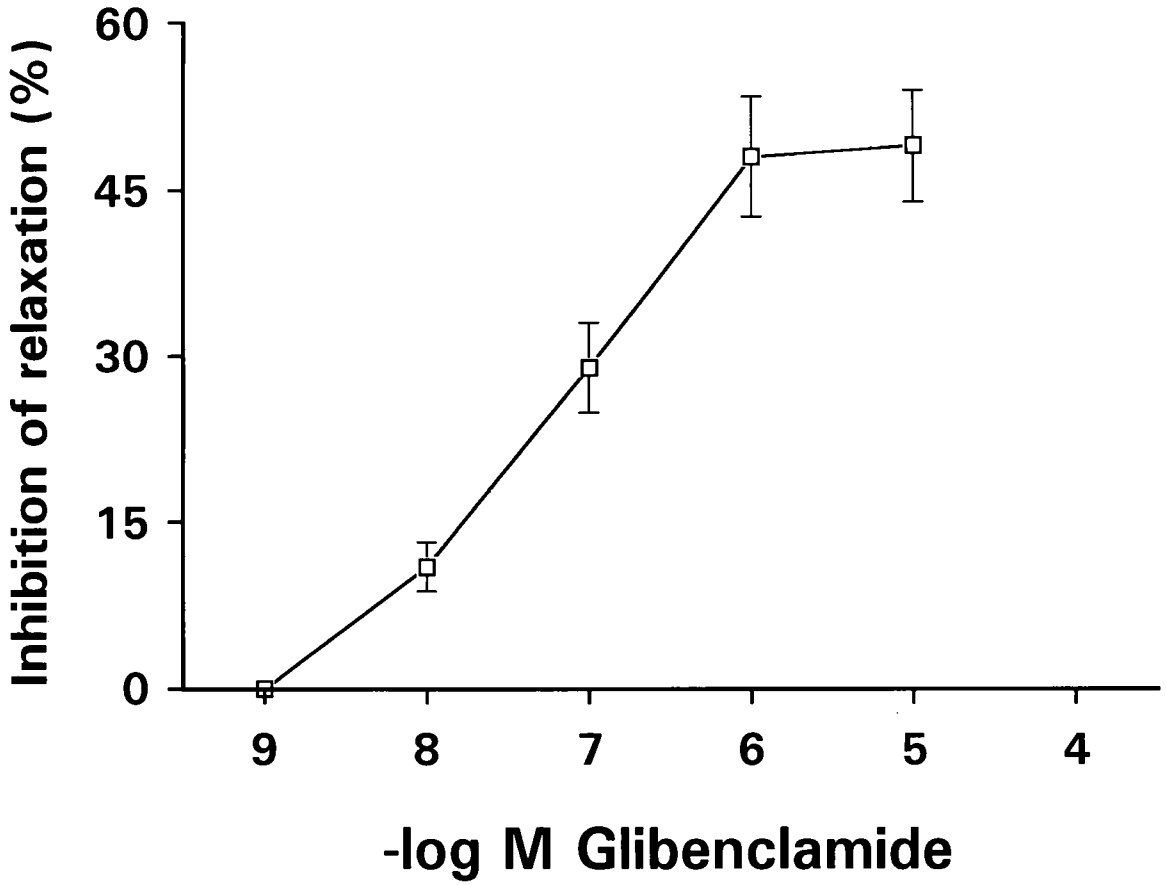
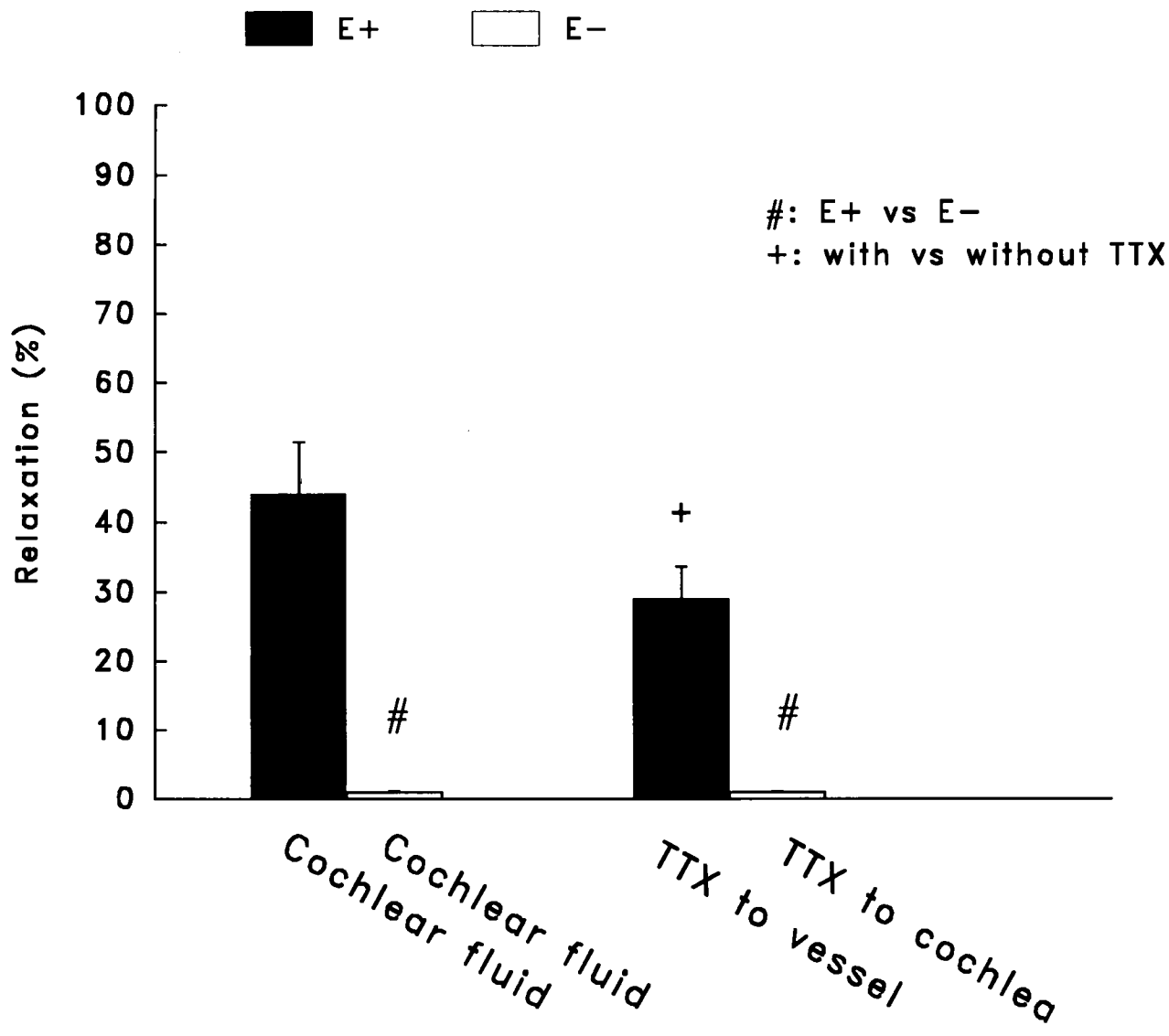
Legends to figures

Figure 1. Endothelium-dependent relaxation by cochlear nerve stimulation of aortic rings of the rabbit. Rabbit aortic rings were exposed to organ fluid of isolated guinea pig cochleas (see methods), which had been subjected to electrical field stimulation (50 V, 50 Hz, 0.1 ms impulse duration). Abbreviations:E+: aortic rings with intact endothelium; E-: aortic rings from which the endothelial layer was gently removed; TTX: 1μM tetrodotoxin. The data are means $\pm$ S.D. obtained with 6 preparations. The level of significance: $p < 0.05$

Figure 2. Glibenclamide inhibits endothelium-dependent relaxation of rabbit aortic rings in response to exposure to organ fluid of the electrically activated cochlea of the guinea pig. A concentration response relationship. The data are means $\pm$ S.D. obtained with 6 experiments.

This work was supported by grants from National Scientific Research Founds (OTKA F19946 and D23736) and National Techical Development Commitee (OMFB 05201950616), and the Hungarian Space Research Office, Budapest, Hungary.


Inhibition of relaxation (%)
60
45
30
15
0
9
8
7
6
5
4
-log M Glibenclamide


E+
E−
100
90
80
70
60
50
40
30
20
10
0
Relaxation (%)
#: E+ vs E−
+: with vs without TTX
+
#
#
Cochlear fluid
Cochlear fluid
TTX to vessel
TTX to cochlea

Milan, July 7, 1998

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Dear Dr. Szilvassy,

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