

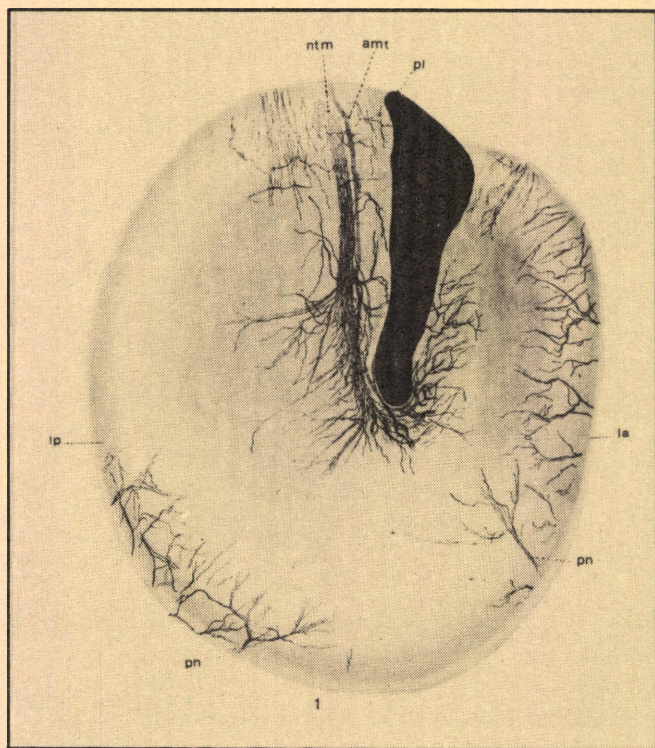
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Topical Anesthesia of the Tympanic Membrane

An experimental animal study

by

Sten-Hermann Schmidt



Tokkopa ketkään hoitavat korvien märkäpesäkkeitä turmiollisesti onnen kaupalla(kuten tavallisesti käy)? Onko parempi odottaa rumpukalvon märkimistä kuin sen käydessä kypsäksi tehdä tie sisäkorvassa pesivälle mädälle ja nesteelle?

J.Busson

käänn.: H Halén

Topical Anesthesia of the Tympanic Membrane

An experimental animal study

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STEN-HERMANN SCHMIDT

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ABSTRACT

TOPICAL ANESTHESIA OF THE TYMPANIC MEMBRANE *An experimental animal study*

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Myringotomy plays an important role in otological therapy. The procedure requires an efficient anesthesia, which can be obtained without general anesthesia. However, the use of local anesthetics on the tympanic membrane (TM) has been abandoned in many places, as general anesthesia has been readily available. In the present study the effects of some commonly used topical anesthetics on the TM structure and inner ear were tested in an animal model (rat and guinea pig).

Four different anesthetic compounds—Xylocaine®, Bonain's liquid, phenol and Emla®—were applied to the TMs of the animals, which were sacrificed 10 minutes to 5 months after application. Morphological effects regarding time after treatment and number of applications were elucidated. At sacrifice the tissue was fixed and the TMs analysed by light microscopy (LM) and transmission electron microscopy (TEM). In nine animals phenol, Xylocaine® Spray or Emla® was applied to the round window niche and ABR recordings were made at 24 h to 6 months after exposure. After the final ABR evaluation the animals were sacrificed and the cochleae prepared for LM and scanning electron microscopy (SEM).

On the TM phenol and Bonain's liquid caused instant destruction of the keratinizing stratified squamous epithelium followed by long-lasting hyperplasia of this epithelium and the underlying connective tissue. A pronounced hyperplasia of these two layers was also noted for the Xylocaine® Spray group, but without immediate destruction of the keratinizing epithelium. The extent of structural changes differed in relation to the extent of spreading of the agent. Emla® showed little, if any, sign of epithelial reaction and had no effect on the connective tissue. Regarding the inner ear Emla®, Xylocaine® Spray and phenol induced significantly impaired ABR thresholds mainly affecting the higher frequencies. However, the impaired ABR thresholds were reversible and at the end of the experiment there was no significant impairment compared to the control data. All agents, except Xylocaine®, damaged the hair cells in the basal part of the cochlea as shown by cytochleogram and SEM analysis.

Instant destruction of the epidermis seems to be necessary for an instant anesthetic effect. All agents caused profound connective tissue reactions. The manner of application, depending on the physical properties of the agent, determined the extent of the structural changes. The changes of the connective tissue were concentrated to the submucosal layer, which seems to be the area for reconstruction of the damaged TM. All agents caused functional inner ear changes. With the exception of Xylocaine® they also caused morphological alterations of the cochlea. The functional changes were partly reversible. Topical anesthetics applied to the TM should be used with caution and when used in an appropriate manner they can be considered safe, especially in an inflamed middle ear, with a thickened round window membrane, which should prevent the agents from reaching the inner ear structures.

Key words: Topical anesthesia, middle ear, tympanic membrane, cochlea, collagen fibres, lidocaine, 96 % phenol, Bonain's liquid, auditory brainstem response, ototoxicity, cytochleogram, scanning electron microscopy, rat, guinea pig.

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University of Umeå, Umeå, Sweden

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Sten-Hermann Schmidt

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Num autem quicumque aurium abscessibus laborant infelici suae
forti (ut fieri solet) permittendi? An praestat membranae tympani
suppurationem expectare, quam, illam mature inciendo, puri aut hu-
moribus in concha interna nidulantibus viam facere?

Juliano Busson M.D. Parisiis 4 Aprilis anno 1748

ORIGINAL PAPERS

The present thesis is based on the following publications, which will be referred to by their Roman numerals.

- I. Schmidt S-H, Hellström S, Carlsöö B. Short-term effects of local anaesthetic agents on the structure of the rat tympanic membrane. Arch Otorhinolaryngol (1984) 240:159-166.
- II. Schmidt S-H, Hellström S, Carlsöö B. Fine structure of rat tympanic membrane after treatment with local anaesthetics. Acta Otolaryngol (Stockh) 1986; 101:88-95.
- III. Schmidt S-H, Hellström S. Late effects of local anesthetics on tympanic membrane structure. Am J Otolaryngol 7:346-352, 1986.
- IV. Schmidt S-H, Hellström S, Anniko M. A new topical anesthetic - lidocaine-prilocaine mixture (Emla^R) - for use in TM anesthesia. Accepted for publication in Arch Otorhinolaryngol.
- V. Schmidt S-H, Anniko M, Hellström S. Effects on the inner ear of the clinically used local anesthetics Xylocaine^R, Emla^R and phenol. Submitted for publication to Am J Otolaryngol.
- VI. Anniko M, Schmidt S-H. The ototoxic potential of Emla^R. A new local anesthetic for the tympanic membrane? Acta Otolaryngol (Stockh) 1987; (in press).

List of abbreviations:

ABR	Auditory Brainstem Response
EAC	External Auditory Canal
HC	Hair Cell
LM	Light microscopy
MEC	Middle Ear Cavity
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TM	Tympanic Membrane

"Soll man die an Ohrenentzündung leidenden ihrem Schicksal (wie es häufig geschieht) überlassen? Ist es besser, die Eiterung des Trommelfells abzuwarten, oder soll man es zeitig genug operieren, entweder solange es rein ist, oder, indem man dem Eiter der sich im Innenohr gesammelt hat, den Weg frei macht."

Juliano Busson M.D. Paris, 4 April 1748

Übers.: J.G.Stipa

Skall man då överlämna alla som plågas av varhård i öronen åt sitt olyckliga öde (såsom brukar ske)? Eller är det bättre att invänta trumhinnans bulnande än att, skära i den när den är mogen och skapa en väg för det var och de vätskor, som finns i mellanörat?

övers.: S.Jacobsson

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INTRODUCTION

The first documented procedure recognizable as a paracentesis of the ear drum, or myringotomy, was performed in 1760 by Eli in Paris, as reported in a letter by Gottlieb Emanuel von Haller in 1760 (1). Most medical historians, however, credit Sir Astley Paston Cooper, surgeon at Guy's Hospital (London), with the introduction of myringotomy in 1800 (2). However, not being aware of each other and probably earlier than Cooper, Peter Degrauers a contemporary doctor in Edinburgh, who styled himself professor of anatomy and physiology, also performed myringotomies (3).

Cooper advocated myringotomy for cases of deafness due to a blocked Eustachian tube. His opinion was strongly supported by Himly of Göttingen (4). In France, Portal favoured myringotomy for cases of thickened membranes (5), whereas the suggestions of Busson (6) and Alard (7) to open the tympanic membrane (TM) to evacuate inflammatory - especially suppurative - material, did not gain any adherents at that time.

Myringotomy attracted increasing interest and soon the procedure was frequently performed in Germany (Michaelis, Hunold), in France (Mannoir, Celliez, Saissy), in Britain (Saunders, Travers) and in Italy (Fabrizi) (8). Myringotomy was often described as a painful procedure but none of the reports from this era mention any anesthesia in connection with the surgical intervention. The human TM has a sensory innervation with free endings lying near the surface between the epithelial cells (9,10). The slightest touch of its surface can cause painful - though brief - reactions.

The use of local anesthesia in otology, especially for myringotomy, began in the 1880s. It was firstly described to the German Medical Society in Prague on November 14, 1884, when Emanuel Zaufal announced the use of 10% alcohol-cocaine solution on the ear drum (11). Cocaine, however, seemed to be used only occasionally for ungovernable children and unusually sensitive adults. Not even the efficacy of a 20% solution was known to be reliable, after being in contact with the TM for more than 5 min (12).

In 1898 Bonain introduced his liquid, which is a eutectic mixture of equal parts of phenol, menthol and cocaine (13). The efficacy of Bonain's liquid is attested by the fact that, from its introduction to the present time, it has probably been the most commonly used agent for topical anesthesia of the TM (14-17). Many other mixtures have been introduced in attempts to improve on Bonain's original liquid. Some of these are: the mixture recommended by Hechinger: acid carbolic liquefact. 0.5, menthol, cocaine. muriat. aa gmta 2.0, spirit. conc. ad gm. 10 (18), Gray's fluid consisting of 5-20% cocaine in equal parts of absolute alcohol and aniline oil (19) and Blegvad's solution containing equal parts of cocaine hydrochloride and salicylic acid and two parts of rectified spirit (20). Stout replaced cocaine in Bonain's liquid with clove oil (21). A review of the various mixtures is listed by Uhde (22).

In 1956 Lloyd Storrs introduced pure phenol as a topical anesthetic agent for the TM. He had found that phenol was a common component in a variety of mixtures used for topical TM anesthesia. He inferred, as did Freystadt as long ago as 1934 (18), that phenol could be the primary anesthetic agent of these drugs. It seemed to embody two steps in its anesthetic action; it coagulated the epithelium, exposing the sensory nerve endings, which allowed the chemical to exert its anesthetic properties (23). According to extensive experience, Bonain's liquid and pure phenol are both potent anesthetics for the TM. Both agents have been frequently used, but some questions about their adverse effects have emerged, however, especially concerning their caustic properties (20,22,24,25). In these cases the adverse effects have been caused by an incorrect handling of the drug.

Histological architecture of tympanic membrane

In man, as in several animal species, the pars tensa of the TM is built up of five different layers. On the outer meatal side there is an epidermal layer consisting of keratinizing, stratified squamous epithelium, three to seven cells in thickness. Underlying the epithelium there is a thin connective tissue layer. Thereafter follows a dense layer consisting predominantly of collagen fibres

arranged in a typical pattern of inner circular and outer radial fibres. Finally, facing the ear cavity there is a thin layer of loose connective tissue covered by a single layer of flattened epithelial cells.

In this study the different TM layers are named according to the nomenclature proposed by Lim (26).

OBJECTIVE

The question of the adverse effects of topical anesthetics has arisen repeatedly (27-29). Phenol is a caustic agent and it is for instance well known that a casual touch of the external auditory canal (EAC) with phenol-soaked cotton will invariably be painful and cause an external otitis. Particularly it has been feared that phenol and Bonain's solution could create permanent perforations if applied on atrophic membranes.

Surprisingly the documentation of effects of local anesthetics on TM and the middle ear structures is meagre. Ikebe (30) focused on the effects on the EAC after exposure to Novocaine, while Pizzolato and Mannheimer (31) concentrated on phenol injections into neural tissue.

The objective of the present study was to investigate the histopathological effects of the topical anesthetics, Xylocaine^R, Bonain's liquid, phenol and Emla^R on the middle ear, particularly the TM, and the inner ear, in an animal model (rat and guinea pig).

Specific attention was paid to:

- (a) the immediate effects of various topical anesthetic agents on the TM structure (I),
- (b) fine structural analysis of the processes of epithelial destruction and repair induced by topical anesthesia on the TM (II)
- (c) the long-term effects of topical anesthesia on the connective tissue of the TM (III)
- (d) the effects of a new topical skin anesthetic - the eutectic

lidocain-prilocain mixture - Emla^R on the TM structure (IV) and the cochlea (VI), and

(e) the effect on inner ear structure and function after maximum exposure of the middle ear cavity (MEC) to TM anesthetics (V).

MATERIALS AND METHODS

Altogether 120 healthy Sprague-Dawley rats and 30 guinea pigs were used for the studies. In the initial experiments the desire was to mimic the clinical situation (I, II, III, IV) whereas the studies on ototoxicity (V, VI) required maximum exposure of the cochlea. The latter effect was achieved by placing crystalline phenol (V) in the round window region or by filling the round window niche with Xylocaine^R Spray (V) or Emla^R (V, VI). The studies on Emla^R also included repeated applications of the active substance as well as its solvent.

In order to facilitate application of drugs and inspection of the TM status by otomicroscopy, the rats were anesthetized with sodium hexobarbital i.v. through a tail vein. For similar procedures the guinea pigs were subjected to anesthesia by inhalation of ether in a sealed cage.

Local anesthetics used

Phenol 96%, Bonain's liquid, Xylocaine^R Spray, Emla^R, Xylocaine^R 4% and physiological saline, were used for application on the TM surface.

Phenol or carbolic acid occurs in crystalline form with a purity of 96% and with a melting point of 41°C when free from water. It is liquefied by adding 8% water.

Bonain's liquid is a eutectic mixture of equal parts of phenol, menthol and cocaine hydrochloride.

Xylocaine^R Spray is composed of lidocaine 10 mg, cetylpyridine chloride, aethanol, macrogol, aroma, trichlorfluormethane et dichlorfluormethane q.s.

Emla^R is composed of 25 mg lidocaine and 25 mg prilocaine per gram

and polyoxyaethylene, carboxypolyethylene, sodium hydroxide, aq purif q.s. 1 ml

Xylocaine^R 4% consists of lidocaine hydrochloride anhydride 40 mg, sodium hydroxide, preservative (Metagin), purified water q.s.

Techniques for application

Depending on the physico-chemical properties of the anesthetic agents, the application of each substance was performed with different techniques. Application of phenol on the TM was performed with a micropipette (I) or by touching the TM surface with a soaked cotton swab (III). Bonain's liquid was pipetted onto the TM surface (I) or applied by means of a soaked-cotton ball (III), which was left for 5 min on the TM surface. Xylocaine^R Spray (I,II,III) and Xylocaine^R 4% (I) were both pipetted onto the TM surface. Emla^R was applied with a 0.7 mm cannula to cover the TM (IV).

In preparation for ABR recordings(V), the upper rear quadrant of the left ear was perforated to facilitate application of the anesthetics in the round window area. This was accomplished by means of a micropipette for Xylocaine^R Spray and Emla^R. The phenol crystals were deposited by means of a suction device.

Morphological studies

The follow-up times from exposure to sample collecting of TMs were 10 min, 24 h, 1 week and 2 weeks (I); 10 min, 24 h, 1 week, 2 weeks and 3 weeks (II); 2 months and 5 months (III); 2 weeks and 2 months after a single application and 2 weeks and 4 months after the last of the multiple applications (IV); 6 months (V); 3 weeks after the last application into the MEC and 2 weeks and 6 weeks respectively after single and multiple administration into the EAC (VI).

At sacrifice the rats were reanesthetized and then fixed by perfusion with 2% glutaraldehyde solution in cacodylate buffer through the left heart ventricle. The pars tensa of the TM was then dissected free in one piece from the sulcus tympanicus and processed

for light microscopy (LM) (I, II, III) and transmission electron microscopy (TEM) (II, III, IV). The TMs were rinsed in buffer, then postfixed in 1% OsO_4 in cacodylate buffer. After dehydration in increasing concentrations of ethanol the specimens were embedded in Epon 812. Semithin ($0.5\ \mu\text{m}$) sections were cut on a Porter-Blum ultramicrotome, stained with toluidine blue and then examined under a light microscope or thin sectioned ($70\ \text{nm}$) for ultrastructural analysis.

The guinea pigs were anesthetized with ether, decapitated and then immersion-fixed before dissection of the TM (IV), which was carried out by resecting the entire TM out of the sulcus tympanicus. The TMs were prepared as described above for the rat TMs.

For the inner ear morphology the cochleae of the rat (V) and guinea pig (VI) were removed from the skull base and immersed in 3% glutaraldehyde. After postfixation in 1% osmium tetroxide the specimens for LM were decalcified in an EDTA solution (32). Following dehydration in alcohol and embedding in an Epon mixture the cochleae were divided in a mid-modiolar plane and each half serially sectioned. The sections were stained with toluidine blue before examination. For scanning electron microscopy (SEM) (VI) the postfixed and dehydrated specimens were prepared by the OT0 technique. Prior to critical point drying, the bone of the whole cochlea was drilled away and after drying, the specimens were covered with gold and then examined.

ABR analysis

ABR recordings (V) were performed before exposing the ear to the various anesthetics prior to and after the myringotomy. After application of each local anesthetic ABRs were recorded at 24h, 3 weeks, 2 months and 6 months.

In the anesthetized animals, three needle electrodes were inserted through the skin: one at the vertex (reference electrode), one in the left brachial region (positive electrode) and one into the right brachial region - a grounding electrode. The animals were held prone in an acoustically shielded box on a thermostatically

controlled heating pad, keeping a temperature of $37.0 \pm 0.2^{\circ}\text{C}$. The auditory stimuli were delivered via a Beyer TDL telephone, fitted into the EAC by an ear speculum. The stimulus was a full cycle tone burst at a fixed frequency from 2 kHz to 31.5 kHz, presented at a rate of 16/s. The rise and fall times were 1 ms each, with a total exposure time of 5 ms. For each measurement, 1024 epochs were stored and presented on a storage oscilloscope and recorded on a plotter. An artifact rejection unit prevented analysis of electroencephalogram potentials exceeding 20 μV . The ABR thresholds were defined as the lowest value at 5 dB attenuator steps, giving reproducible answers. The cochlea of the contralateral ear had been mechanically destroyed one week before the start of the experiment.

RESULTS

I. Short-term Effects on TM Structure of Topically Applied Local Anesthetics

The effects of phenol, Bonain's liquid, Xylocaine^R Spray, Xylocaine^R 4%, and NaCl were compared at 10 min, 24 h, 1 week and 2 weeks, on the TM structure.

Ten minutes after application of phenol an ablation of the epidermis occurred at the area of contact with the phenol drop. An ablation of the mucosal lining was seen at the corresponding site. At 24 h, swelling of the fibrous layer and invasion of inflammatory cells were noted, whereas hyperplasia, mainly involving the epidermis, occurred after one week. The hyperplasia was most accentuated around the denuded areas. After 2 weeks the hyperplasia was reduced but still present in all layers. Inflammatory cells were no longer observed.

Bonain's liquid had caused effects similar to those of phenol noted at 24 h and showed disjunction or total loss of the epidermis and swelling of the fibrous layer with inflammatory cells attached, replacing large areas of the mucosal lining. At one

week, swelling and hyperplasia were the main features of all layers, together with an inflammatory reaction in the mucosal layer. Denuded areas of the fibrous layer were still present on both the inner and outer surfaces. After 2 weeks the thickness was reduced, but swelling and proliferation of epithelia and connective tissue still persisted. The occurrence of inflammatory cells had ceased.

Ten minutes after application of Xylocaine^R Spray the TM was swollen, with increased desquamation of the epidermal layer. The swelling was further enhanced at 24 h and in some areas the epidermis was lacking. Inflammatory cells were present in the mucosa. After one week a magnificent hyperplasia of the epidermal layer was present, and the inflammatory reaction had increased. Even at 2 weeks the hyperplasia persisted to almost the same extent.

Xylocaine^R 4% caused only slight swelling of the entire TM and sparse submucosal inflammation at 24 h. At 1 week an increased epithelial desquamation was seen.

TMs treated with NaCl did not show any changes, compared with untreated controls.

II. Changes of Fine Structure of TM after Xylocaine^R Spray Application

Ten minutes after application of Xylocaine^R Spray, TEM analysis disclosed necrotic and severely damaged epithelial cells in the epidermis. Destroyed cells were also detected in the mucosal epithelium. After 24 h the destruction of the epidermis was further enhanced. The mucosa was partly lacking, and exposed bare collagen bundles towards the tympanum. In some areas the denuded fibre layer was covered by inflammatory cells, mainly polymorphonuclear neutrophils.

After one week the epithelial layers showed hyperplasia with an increased cell size and an increased number of cell layers and

widened intercellular spaces. In the fibrous layer the number of fibroblasts were increased. Granulocytes were infiltrating in between the swollen mucosal cells. After 2 weeks most of the drum contained connective tissue, with many fibroblasts. The connective tissue was covered by a hyperplastic epidermis. The mucosal epithelium was cuboidal and contained lymphocytes, granulocytes and macrophages. After 3 weeks the epidermis was almost normalized, though still exhibiting an increased desquamation. The connective tissue layer dominated the TM, with many fibroblasts towards the tympanic side. The mucosal epithelium was completely normalized.

III. Long-term Effects after Exposure of TM to Local Anesthetics

The observation time after exposure to Xylocaine^R Spray, Bonain's liquid and phenol was extended to 2 months and 5 months. At 2 months after treatment with Xylocaine^R Spray the fibrous layer was excessively thickened and exhibited many fibroblasts and a loose collagen network towards the tympanic side. The epithelial linings were normal. At 5 months the changes had diminished, though the fibrous layer was still thickened, with many fibroblasts and newly formed fibres still lacking strict bundling.

Two months after exposure to Bonain's liquid the thickening of the TM was restricted to that part of the TM which had been exposed to the anesthetic. Signs of new connective tissue being formed on the tympanic side of the fibrous layer were seen. At 5 months the structure was almost normalized, although the TM still showed a noticeable thickening.

Two months after exposure to phenol the TMs exhibited a well defined thickened area, where newly formed connective tissue predominated. The epithelia in these areas also showed swollen cells. After 5 months these changes were markedly reduced, but still discernible. Statistical comparison of the anterior and posterior TM halves revealed a diffuse thickening of the drum after Xylocaine^R Spray and Bonain's solution, whereas after phenol the thickening was restricted to the treated posterior half (Fig. 1).

MEDIAN VALUES OF TYMPANIC MEMBRANE THICKNESS

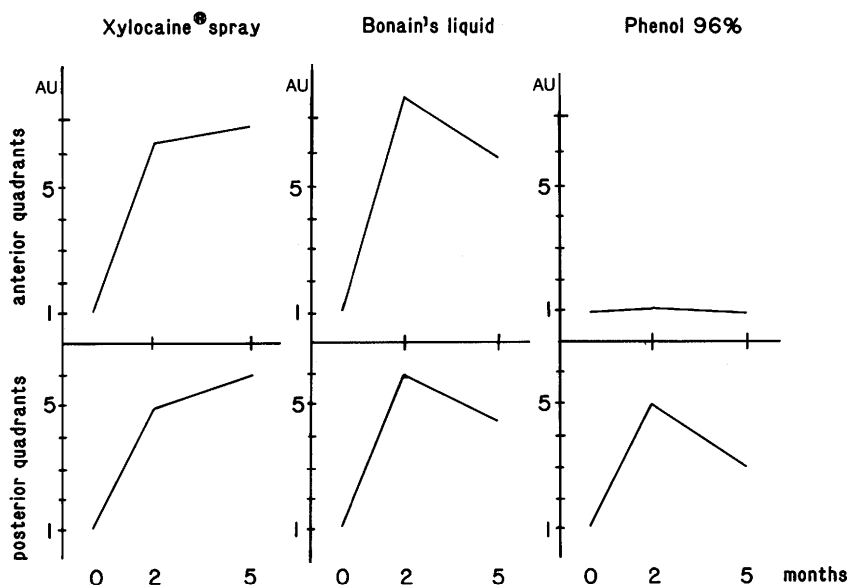


Fig. 1. Median values (arbitrary units = A.U.) of TM thickness of the anterior and posterior quadrants at 2 and 5 months after application of the various anesthetics to the posterior quadrant.

IV. Effects of Emla^R on TM Structure

Application of Emla^R on the TM caused an increased production of wax in the EAC of both rats and guinea pigs. Dilated vessels were regularly noted in some of the placebo (solvent of Emla^R) treated ears, but also in a few Emla^R-treated ears. A whitish colouring with reduced translucency of the TM were evident in half of the cases after one week. All Emla^R-treated TMs were affected with this discolouring at the time of the fifth application. In the placebo-treated rat TMs, only two showed a reduced translucency and none of the guinea pig ears exhibited this sign. In the light microscope an increased desquamation of the keratinizing squamous

epithelium was the dominant feature. A slight swelling and occurrence of inflammatory cells were also present, but these changes diminished with extended observation time. The placebo-treated rat TMs were normal. Nor did the TMs of the Emla^R-treated guinea pigs differ from the placebo-treated and the untreated specimens.

TEM analysis revealed a moderate swelling of the connective tissue after a single application of Emla^R and 2 weeks of follow-up. On extending the observation period to 2 months, the changes declined considerably. TMs treated repeatedly (five times at weekly intervals) showed alterations less pronounced than those followed for 2 weeks after a single application. Except for a minimal submucosal edema the placebo-treated TMs appeared normal.

Guinea pig TMs after a single treatment showed minor submucosal edema, whereas the TMs after repeated treatment exhibited quite normal features.

V. Local anesthetics applied in the round window niche

Morphological observations of the EAC and MEC. Perforation of the upper rear quadrant caused dilated vessels along the malleolar shaft. After exposure to the three agents, changes developed which were specific for each group. 24 hours after application of Xylocaine^R Spray, a fibrin plug clogged the EAC. The MEC was aerated, but veil-like structures (fibrin?) crossed the cavity. At the same time Emla^R had led to heavy epithelial desquamation and accumulation of clear fluid in the EAC. Phenol had induced an external otitis with narrowing of the EAC. Wax and detritus covered the TM. At 3 weeks the TMs of the Xylocaine^R Spray group were healed, with an opacity at the perforation site. In the Emla^R group the perforations were still open or else clogged with wax. The phenol group exhibited a narrow EAC with wax and detritus and an aerated MEC. After 2 months the manubrial vessels were still dilated in all groups. Opacity of the TM was found in the Xylocaine^R Spray and Emla^R groups. In the phenol group the EAC was still narrow and wax obscured parts of the TM, but at least one TM

perforation was closed. At 6 months the only notable finding was a persistent reduction of the ear canal diameter (about 20%) in the phenol-treated group.

ABR observations. With the ABR technique an electrophysiological threshold can be measured and its changes recorded (33). The ABR analysis revealed that all agents tested had induced a pronounced impairment of the threshold 24 h after application in the round window niche area (Fig. 2). For Xylocaine^R it affected all frequencies, showing a significant impairment of thresholds at 8, 12, and 16 kHz, with a maximum change at 12 kHz. At 3 weeks the ABR thresholds in the Xylocaine^R-treated ears had returned to normal, except for a significant impairment at 2 and 20 kHz only. Compared with the thresholds at 24 h, the ABR thresholds had improved significantly at 20 kHz. At 2 months the Xylocaine^R-treated ears only showed a significant ABR threshold impairment at 16 and 31.5 kHz. Six months after Xylocaine^R treatment, a significant ABR threshold impairment was noted at 12 and 31.5 kHz. A significant improvement compared with the control level was noted at 4 kHz.

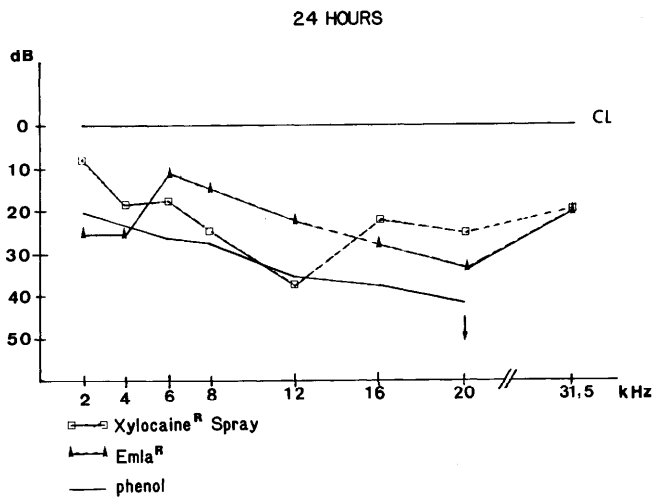


Fig. 2. Mean ABR thresholds at 24 h after application of the three local anesthetics compared to the control (CL) level.

After 24 h, Emla^R had caused an impaired threshold at 2, 4, 12, 16, 20 and 31.5 kHz with a maximum change at 20 kHz. Even 3 weeks later the ears showed impairment at 8, 16, 20 and 31.5 kHz. At 4 kHz a significant improvement above the pre-treatment level was observed. At 2 months, Emla^R-treated ears exhibited no significant ABR threshold change. The improvement at 4 kHz still persisted at 6 months (Fig. 3).

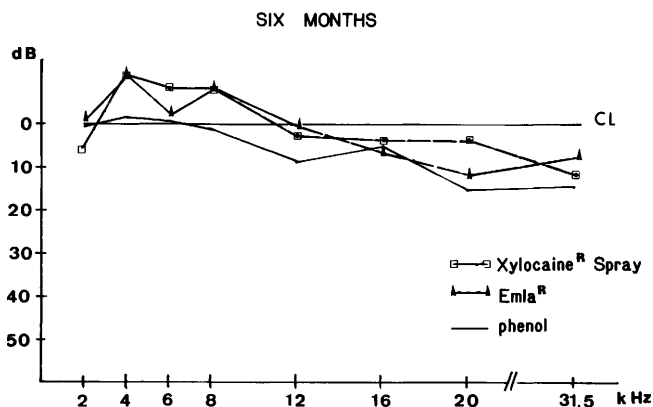


Fig. 3. Mean ABR thresholds at 6 months after application of the three local anesthetics compared to the control (CL) level.

The phenol-treated ears exhibited a sloping curve, with an increasing impairment of threshold towards the higher frequencies. The alterations after phenol exposure were statistically significant at all frequencies except 4 kHz. The impaired thresholds persisted at 16 and 31.5 kHz after 3 weeks. At 2 months the phenol-treated ears showed improved thresholds at all frequencies, but the threshold impairment was still significant at 16 and 31.5 kHz. At 6 months the phenol-treated ears only had a significant impaired threshold at 12 kHz.

The latencies of wave II at the ABR threshold were significantly prolonged at 2, 4 and 12 kHz 24 h after exposure to Emla^R and at 2 kHz 24 h after phenol administration.

Inner ear morphology. Structurally the vestibular organs were well preserved in all groups, whereas the organ of Corti was damaged by phenol and Emla^R. These anesthetics caused morphological alterations with the same general features but to different extent along the basilar membrane. The most extensive changes were elicited after application of phenol. Outer hair cells were more vulnerable than inner hair cells.

The stria vascularis, the spiral prominence and Reissner's membrane were intact.

In one phenol-exposed labyrinth connective tissue-like material was detected in the scala tympani and the scala vestibuli at the basal hook level. At this level also degeneration of most spiral ganglion cells had occurred.

Xylocaine^R Spray did not affect the cochlear morphology.

VI. Effects of Emla^R on Inner Ear Morphology

Irrespective of the mode of application, exposure of the guinea pig inner ear to Emla^R produced clinical reactions emanating from the vestibular system. These reactions declined with repeated applications.

In the guinea pig, application of the solvent of Emla^R into the MEC caused derangement and even a complete loss of all sensory hairs of individual hair cells (HC) in the first few millimetres from the round window. According to the cytochleogram technique, no morphological difference was noted, whether the agent was administered once or repeatedly. Emla^R caused structural changes of the cochlea within 4 mm from the round window. Of these the first 2 mm showed total damage of the organ of Corti. The next 2 mm exhibited destruction of HC, leaving the supporting structures preserved. At 3-4 mm from the round window the inner HC were present, while the outer HC were destroyed. The demarcation was very sharp between normal and completely destroyed cochlear structures. With SEM, derangements of stereocilia were revealed at an additional

distance of 1-2 mm. After repeated exposure, the changes also engaged the third coil, comprising the third and second row of outer HC. When HC were present the sensory hairs were often severely distorted or even absent. Vestibular reactions did not appear after application into the EAC. Nor did application into the EAC in any way alter the cochlear morphology.

DISCUSSION

Various methods, ranging from complete general anesthesia to sedation of varying degrees and local anesthesia, have been used to avoid the pain associated with myringotomy. Local anesthesia has been induced either by infiltrating injections of different anesthetic substances or by topical application of anesthetic agents directly onto the TM. In order to find an optimal method, certain features have to be taken into account. The method should be simple, effective, and harmless without any adverse effects. It is also an advantage if the cost of the procedure, in the form of personnel and the time required, can be kept to a minimum. Not least, the anesthesia should match the duration of the surgical procedure.

Indications for local anesthesia

Myringotomy may be performed for a variety of reasons, whether therapeutic or diagnostic. The use of myringotomy to treat inflammatory conditions involving middle ear fluid is well known. If the old rule, ubi pus, evacua, (wherever pus, remove it) is accurate (the introduction of antibiotics should not modify this principle), it will certainly apply to the MEC, which is situated near to various vulnerable structures including the delicate sensory organ. This increases the possibility of undesirable effects exerted by any pathological content of the middle ear (34,35). It can be concluded from many investigations that an increased use of myringotomy will favour the healing of inflammatory conditions in the middle ear. It is known for instance that when used in an early stage for treatment of acute otitis media, it shortens the

period of recovery and reduces the number of patients developing secretory otitis media (SOM) (36), and most probably also other serious complications (37) including sensory neural hearing loss (38). Our increasing knowledge of possible deleterious effects of endotoxins on the MEC (39) and the cochlea (40,41) also makes early evacuation of the MEC beneficial. Against this background, any unnecessary delay, often amounting to weeks, to await general anesthesia must be considered a disadvantage. Regarding general anesthesia it should also be noted that this procedure per se can have adverse effects on the hearing (42).

Anesthetic methods

Methods with topical application of small quantities of drugs directly onto the TM have unquestionable advantages in contrast to procedures involving local infiltration by injection. The latter is a considerably more complicated procedure and additionally carries a small though real risk of infection, and the procedure is often as painful as the surgical pain it is designed to alleviate.

The topical anesthetics used have, however, different characteristics, e.g. regarding the wide range of time intervals needed to induce anesthesia. Direct application of phenol at the myringotomy site, instantly induces an effective anesthesia of that area, whereas Bonain's liquid applied similarly requires up to 10 min to induce adequate local anesthesia. An alternative method for topical anesthesia is iontophoresis, but this is a rather complicated and time consuming procedure (43) and in any case gives unreliable anesthetic results (44). Additionally complications have been reported, such as facial paralysis (45,46). Despite frequent use since their introduction for topical purposes on the ear drum, very few adverse effects have been reported in connection with Bonain's liquid and phenol, and invariably when incorrectly applied (24,25). When used in an unsuitable manner, all topical anesthetics, including iontophoresis, can affect the vestibular and cochlear functions (14,45). A disciplined exercise of any anesthetic method is a precondition for its safe use. The most suitable way to apply phenol to the TM is to touch the myringotomy site with a slender cotton-tipped applicator, soaked in 96% phenol. At

this concentration, phenol is only partly liquid at body temperature. The use of this concentration in such a minute amount ensures a limited spread and inhibits dripping. This makes it possible to avoid application on the EAC and the occurrence of external otitis but also inhibits application into the MEC. The application of Bonain's liquid should also be submitted to strict rules; a small cotton ball (2-3 mm) is soaked in the highly viscous compound. The wet ball is placed over the site for the planned myringotomy and left for up to 10 min. The present study also supports this rule; if applied to a limited area of the TM structural changes will be minimized.

Structural changes of the TM

With both phenol and Bonain's liquid a whitish opaque spot occurs, which indicates the completely anesthetized area. Regarding this clinical observation it is interesting to note that the present experimental studies concerning structural changes of the TM, induced by local anesthetics such as phenol, revealed destruction of the epidermis. This process happened instantly with phenol and, in contrast, considerably later with Xylocaine^R Spray (I,II). This leads to the proposal that an instantaneous and adequate anesthesia requires removal of the keratinizing squamous epithelium, at least partially, as earlier suggested by Freystadt1 (18), Uhde (15), Ochs (47) och Abramsson (48). These authors stated that most topical agents tried were either caustic or ineffective. One is tempted to suspect the corrosive effect of being essential for its rapid anesthetic action. This event probably uncovers the sensory nerve endings to the attack of the anesthetic substance, which could explain the effectiveness of Xylocain^R Spray in cases of acute otitis media where the epithelial cover is already leaky.

All anesthetics induced a pronounced hyperplasia of the epidermis, which persisted for weeks. The hyperplasia involved the entire external surface of the TM in the case of Xylocaine^R Spray, a limited area with Bonain's liquid and an even smaller area with phenol. The difference in size of the areas affected is partly dependent on the physical properties of each compound, e.g. its viscosity, which is the lowest for Xylocaine^R Spray and

considerably higher for Bonain's liquid. Furthermore, in the case of phenol, the small volume used limits the possibility of spread. The hyperplasia of the epidermis was followed by a hyperplasia of the connective tissue, which persisted throughout the observation period (III). With Xylocaine^R Spray the connective tissue hyperplasia increased between the observations at 2 and 5 months after application, whereas it declined in the same interval after the use of Bonain's liquid and phenol.

The results also suggest that the reconstructive area in an injured TM is located immediately beneath the inner epithelial lining, in the submucosal connective tissue. In this region, an abundance of fibroblasts occurred. The delayed remodelling phase of the connective tissue in the case of Xylocaine^R Spray cannot be explained at present. As the increased volume of the TM will affect the hearing at some point, it can be considered an advantage to use an anesthetic which induces the slightest changes possible.

Inner ear effects of topical anesthetics

The structural studies in (V) and (VI) demonstrated well preserved vestibular organs behind the oval window, while the organ of Corti, in its basal parts close to the round window membrane, exhibited the most pronounced changes, i.e. loss of HCs and even degeneration of spiral ganglion cells. The present electrophysiological studies revealed a marked impairment of ABR threshold upon maximum exposure of the round window area to each anesthetic during the first 24 h (Fig. 2). Surprisingly, functional recovery was demonstrated despite pronounced initial impairment, particularly at the higher frequencies (V) (Fig. 3). One could reasonably suspect a disturbance of the homeostasis of the organ of Corti to be the mechanism involved in this type of temporary hearing loss. Re-establishment of fluid compartments in the organ of Corti seems plausible as also recovery of some stereociliary damage. Reversible functional inner ear changes have recently been described after exposure of the MEC to hyaluronan (49), an endogenous substance lacking toxicity but with osmotic properties.

In the clinical situation a considerable number of ears - with perforated TMs - are treated with locally applied antibiotics, often in combination with glucocorticoids. A number of antibiotics used in chronically discharging ears are well known for their ototoxicity (50). Recent studies have shown that even hydrocortisone may affect inner ear function if applied in the round window niche (51).

The oval and round windows are the least well protected passages between the MEC and the inner ear. The round window is a tiny membrane consisting of a connective tissue layer with an outer layer of simple squamous epithelium and an inner layer of mesothelial cells (52). It can be assumed that the round window is the main gate for ototoxic drug action (53) in an uninfected ear, as the round window membrane is thin in the healthy middle ear.

The adverse effects of the topical drugs may even be exacerbated at a later stage of treatment when a reduced inflammation should give a reduced thickness of the round window membrane. In an untreated purulent otitis media the round window membrane becomes thickened manyfold (54).

Animal model

The rat and the guinea pig are common experimental animals easily obtained and reared. The rat has been suggested to be a suitable model for experimental middle ear research. The gross anatomy of the middle ear of the rat - and especially its mucosal cover - has significant similarities to the human ear (55). The guinea pig, being the classical experimental model for studies on ototoxicity, was included in this study for reasons of comparison of the two species, regarding both the structural findings in the cochlea and the histology of the TM after exposure to the different agents. Secondi (56,57) and Fumagalli (58) described appreciable differences between the human TM and the TM of the guinea pig. The present study revealed distinct differences, too. The structural composition of the guinea pig TM is based on a large-meshed net of gross fibre bundles with loose connective tissue interposed. In comparison, the rat TM exhibits a much more homogeneous fibrous

layer, not organized in bundles except for the circular bundles at the very periphery of the drum. The overall measures of the TMs of the two species differ according to the size of the animals. In fact it could be shown that the architecture of the rat TM more closely resembles that of the human TM (59), than the TM of the guinea pig. Though differing in size, the close resemblance of the human TM and rat TM is an advantage, when comparing effects of different substances or pathologic processes on the TM. The wafer thinness of the rat TM is not necessarily an obstacle in these TM experimental studies, since possible harmful effects should be even more easily revealed.

In this study concerning the inner ear, albino rats were used. One must be aware of a possible influence of pigmentation, as differences occur in the susceptibility of albinos to different ototoxic agents (60,61).

Moreover, rather remarkable vestibular reactions were noted after the first application of Emla^R into the guinea pig ears, whereas no such reactions were noted with the rats. This phenomenon we have so far considered to be related to species differences.

CONCLUSIONS

From the present experimental work it can be concluded that:

(a) Efficient topical anesthetics cause instant destruction of the keratinizing squamous epithelium. The ablation of the epithelium seems to be a prerequisite for efficient anesthesia to occur. Little or no destruction of the keratinizing squamous epithelium suggests a slow-acting anesthetic.

(b) The extent of the structural changes of the TM differs according to the physical properties of the agents; if the drug is applied to small areas, the least widespread changes will occur.

(c) Xylocaine^R Spray does not cause any immediate destruction of the keratinizing squamous epithelium. Nevertheless it causes the most extensive epithelial changes and the most extensive and long-lasting changes in the connective tissue.

(d) In general, the effects of topically applied anesthetics on the different TM layers occur in the following chronological order: destruction of the keratinizing squamous epithelium, followed by a swelling of the connective tissue. The damaged epithelium heals within weeks, whereas the reorganization of the connective tissue takes several months.

(e) The repair process in the connective tissue of the TM appears to involve the submucosal layer, which contains the bulk of the numerous fibroblasts. This region presumably acts as a germinative centre for the renewal of the TM.

(f) When applied to the round window niche, all anesthetic substances tested induced severe impairment of ABR thresholds in the high-frequency range. However, the ABR recovered throughout the study, indicating at least in part a reversible inner ear damage.

(g) When allowed to penetrate the round window membrane, all anesthetics except Xylocaine^R Spray caused severe damage in the basal hook of the cochlea.

(h) Various local anesthetics cause different TM and inner ear effects according to their different properties, physical and other. However, it seems obvious that regarding the extent of morphological changes of the TM, the skill and experience of the physician who handles these drugs are as important as their properties.

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