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Research paper

Cochlear microdialysis for quantification of dexamethasone and fluorescein entry into scala tympani during round window administration

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Abstract

Before new drugs for the treatment of inner ear disorders can be studied in controlled clinical trials, it is important that their pharmacokinetics be established in inner ear fluids. Microdialysis allows drug levels to be measured in perilymph without the volume disturbances and potential cerebrospinal fluid contamination associated with fluid sampling. The aims of this study were to show: (i) that despite low recovery rates from miniature dialysis probes, significant amounts of drug are removed from small fluid compartments, (ii) that dialysis sampling artifacts can be accounted for using computer simulations and (iii) that microdialysis allows quantification of the entry rates through the round window membrane (RWM) into scala tympani (ST). Initial experiments used microdialysis probes in small compartments in vitro containing sodium fluorescein. Stable concentrations were observed in large compartments (1000 μl) but significant concentration declines were observed in smaller compartments (100, 10 and 5.6 μl) comparable to the size of the inner ear. Computer simulations of these experiments closely approximated the experimental data. In in vivo experiments, sodium fluorescein 10 mg/ml and dexamethasone–dihydrogen–phosphate disodium salt 8 mg/ml were simultaneously applied to the RWM of guinea pigs. Perilymph concentration in the basal turn of ST was monitored using microdialysis. The fluorescein concentration reached after 200 min application (585 ± 527 μg/ml) was approximately twice that of dexamethasone phosphate (291 ± 369 μg/ml). Substantial variation in concentrations was found between animals by approximately a factor of 34 for fluorescein and at least 41 for dexamethasone phosphate. This is, to a large extent, thought to be the result of the RWM permeability varying in different animals. It was not caused by substance analysis variations, because two different analytic methods were used and the concentration ratio between the two substances remained nearly constant across the experiments and because differences were apparent for the repeated samples obtained in each animal. Interpretation of the results using computer simulations allowed RWM permeability to be quantified. It also demonstrated, however, that cochlear clearance values could not be reliably obtained with microdialysis because of the significant contribution of dialysis to clearance. The observed interanimal variation, e.g., in RWM permeability, is likely to be clinically relevant to the local application of drugs in patients.

Keywords: Round window membrane; Permeability; Pharmacokinetics; Inner ear; Microdialysis; Perilymph; Drug delivery; Dexamethasone; Steroid

Abbreviations: DexP, dexamethasone-21-dihydrogen phosphate; HPLC, high performance liquid chromatography; RWM, round window membrane; ST, scala tympani; TMPA, trimethylphenylammonium

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1. Introduction

In recent years, an increasing interest in local drug application to the round window membrane (RWM) has emerged. Substances have been shown to enter scala tympani and from there are distributed in the inner ear (Goycoolea, 2001). Recent pharmacokinetic studies have shown that substances applied locally to the RWM produce significantly higher drug levels in the inner ear fluids compared to systemic applications (Bachmann et al., 2001; Chen et al., 2003a; Parnes et al., 1999). Thus, applying drugs locally may avoid potential systemic complications and side effects.

Glucocorticoids have been shown to be able to therapeutically influence various disorders associated with a disturbance of inner ear homeostasis, although the specific mechanisms are not completely resolved (Trune, 2006). There are a growing number of clinical reports with regard to local treatments with corticosteroids in cases of acute hearing loss of various causes (Chandrasekhar, 2001; Gianoli and Li, 2001; Gouveris et al., 2005; Ho et al., 2004; Kopke et al., 2001; Lautermann et al., 2005; Lefebvre and Staecker, 2002; Plontke et al., 2005; Silverstein et al., 1996; Parnes et al., 1999), of Meniere's disease (Itoh and Sakata, 1991; Sennaroglu et al., 2001; Shea and Ge, 1996; Silverstein et al., 1998) and of tinnitus (Cesarani et al., 2002; Coles et al., 1992; Sakata et al., 1997; Shulman and Goldstein, 2000; Silverstein et al., 1996). In addition, a number of investigational drugs show promising results with respect to protection when locally applied to the RWM (Li et al., 2001; Chen et al., 2003b; Hight et al., 2003; Keithley et al., 1998). However, no drugs are yet approved for local application to the round window membrane for a specific inner ear indication and success rates in clinical reports vary considerably.

Before new drugs can be approved for clinical use, preclinical studies are necessary. This includes determining the time course and total dose of the drug within the inner ear fluids. A critically important factor in experimental studies of inner ear pharmacokinetics is the method of fluid sampling used. It has been shown that the act of aspirating a perilymph sample can markedly influence the concentration of the drug in the inner ear fluids. As a result, sample concentrations may not always be a good indicator of the drug level at the site prior to sampling (Salt et al., 2003; Salt and Plontke, 2005; Scheibe et al., 1984).

In contrast, with microdialysis it is possible to measure substances without taking fluid samples, which is especially relevant to small volume compartments such as the inner ear. In vivo microdialysis has been used for more than a decade for clinical and preclinical research especially in the central nervous system (Elmquist and Sawchuk, 1997; Ungerstedt, 1991). Apart from its application to neurochemistry and neurophysiology in studying neurotransmitter release, uptake and metabolism, microdialysis has increasingly been used to address pharmacokinetic questions related to drug distribution and metabolism (de Lange et al., 2000; Elmquist and Sawchuk, 1997). In the peripheral auditory system, microdialysis has been used to measure the concentration of neurotransmitters (Hoya et al., 2001; Jager et al., 2000; Matsuda et al., 1998), urea (Hunter et al., 2003) and for pharmacokinetic studies after round window drug delivery of gentamicin (Hibi et al., 2001).

The advantages of microdialysis for pharmacokinetic studies in the inner ear include: (i) the use of repeated measurements allowing the drug time course to be determined, (ii) prevention of artifacts from perilymph volume loss through leaks, and (iii) limited disturbance of perilymph composition due to the low amount of drug recovery.

2. Materials and methods

2.1. Microdialysis

Microdialysis has been applied to a variety of tissues including the measurement of drugs in the perilymphatic space of the cochlea (Hibi et al., 2001; Hunter et al., 2003; Plock and Kloft, 2005). A microdialysis probe consists of an inner tube and an outer tube that has a semipermeable membrane on its distal tip. In an experiment the inlet and the outlet on the proximal end of the probe are connected with tubing to a syringe mounted on a perfusion pump and a collecting vial, respectively. During the perfusion, a physiological buffered salt solution is driven through the inlet into the inner tube, crosses the tip with the dialysis membrane and leaves the outer tube through the outlet. The transport of molecules across the semipermeable membrane of the tip during the perfusion is driven by passive diffusion and can be described by the Fick equation. The molecules within the extracellular fluid diffuse across the semipermeable membrane into the perfusion fluid along their concentration gradient. The perfusion fluid (dialysate) can be collected and analysed to identify or quantify molecules that are present in the extracellular environment. It is also possible however to use this method to deliver substances to the extracellular environment. For that purpose a substance is added to the perfusate and then diffuses through the semipermeable membrane into the surrounding fluids (Plock and Kloft, 2005; Ungerstedt, 1991).

2.2. In vitro studies

Horizontal single chamber tubular compartments of different sizes were used to perform microdialysis experiments in vitro. Acrylic glass reservoirs with volumes of 1000, 100, 10 and 5.6 μl were produced at an in house workshop facility. Using appropriate tubing, the inlet ports of microdialysis probes (CMA/11 14/01/Cuprophane, CMA/Microdialysis AB, Stockholm, Sweden and MAB 4 Cuprophane, TSE, Bad Homburg, Germany) were connected to a syringe pump and the outlet ports connected to a fraction collector. A microdialysis probe was then inserted under microscope and micromanipulator control into the reservoir that was filled with sodium fluorescein (1 mg/ml, Sigma–Aldrich, Taufkirchen, Germany). The linear flow microdialysis probes with a 6-kDa molecular mass cutoff, membrane length of 1 mm and external diameter of 0.24 mm were perfused with sterile phosphate-buffered saline (PBS) at a rate of 2 μl/min (CMA/102 syringe pump). Dialysate samples were collected into plastic vials, using a refrigerated fraction collector (CMA/170) at 8.5 min intervals for up to 68 min. The sampling time interval was dependent upon the combined dead volume of the microdialysis probe, the outlet tubing and the cannula of the fraction collector. Samples were stored at −20 °C for no longer than 14 days before analysis.

2.3. Assay of sodium fluorescein

The concentration of fluorescein in the dialysate samples was assayed using a fluorescence plate reader (Tecan Fluorescence Reader; Tecan Deutschland GmbH, Crailsheim, Germany) at an excitation wavelength of