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Short Communication

Permeability of the n-alkyl p-aminobenzoate esters across the isolated corneal membrane of the rabbit

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SUMMARY

The permeabilities of the rabbit corneal membrane to an homologous series of compounds (the n-alkyl p-aminobenzoate esters) have been determined by an in vitro technique, and a parabolic relationship between corneal membrane permeability and structure (n-alkyl chain length) is reported. This observation is interpreted as resulting from a change-over in the rate-limiting mass transfer step in the transport process, and it is described that the corneal membrane must be treated as a complex membrane, rather than a homogeneous membrane, to resolve the observed permeability behavior.

The significance of the lipophilic character of a drug molecule on drug activity has been widely studied and frequently reported. Excellent reviews have been contributed by Hansch (1969) and Hansch and co-workers (1972, 1973).

Studies relating drug activity and drug structure generally seek to correlate some biological property (a pharmacological activity or a drug transport parameter) with a physicochemical characteristic of the drug, which is invariably its partition coefficient. General use of this structurally non-specific physicochemical parameter, the partition coefficient, involves its correlation with a biological parameter for an homologous series of compounds.

This preliminary communication concerns the permeability of the intact isolated corneal membrane of the rabbit to a series of n-alkyl p-aminobenzoate esters (methyl through n-hexyl). In the experiments conducted corneal membranes were isolated from sacrificed animals and immediately mounted between identical donor and receptor half-cells of a glass diffusion apparatus. The isolated corneas were mounted with their epithelial sides toward the donor half-cells. The membrane preparation procedures, isolation and mounting, were completed within 15 min of sacrificing the animal and the perme-

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ability experiments were initiated immediately following completion of these procedures. Care was taken during the isolation and mounting procedures to maintain membrane integrity relative to membrane surfaces and curvature. The donor phase for each homolog consisted of a saturated solution of the homolog in physiological saline with an excess of solid drug present. This insured a constant concentration, the saturation solubility, of the homolog in the donor phase solution. During the course of an experiment the concentration of homolog in the receptor phase was not allowed to exceed 5% of that of the donor phase. Therefore, sink conditions prevailed throughout the course of an experiment and adjustments for back-flux were unnecessary. Since the donor phase contained a constant concentration of homolog in solution, the saturation solubility, and sink conditions prevailed, a constant concentration gradient of homolog was maintained throughout the time course of an experiment. The donor and receptor phases were stirred to affect homogeneity of the phases.

The choice of employing isolated and mounted corneal membranes in these experiments, rather than conducting in vivo absorption studies on the intact or whole animal, enabled membrane transport characteristics to be obtained in the absence of precorneal effects, such as protein binding (Mikkelson et al., 1973a, b), instilled fluid drainage and normal lacrimal fluid turnover (Chrai et al., 1973) and in the absence of postcorneal factors, such as the processes of elimination of drug from the aqueous humor. However, results from these in vitro transport studies will later be correlated with in vivo studies using unanesthetized animals, wherein all normal physiological variables will be operative.

Employing the above described experimental approach and by monitoring the appearance of homolog in the receptor phase as a function of time, data typical of a transport process driven by a constant gradient was generated. Namely, a diffusional lag time was observed corresponding to a build-up and saturation of permeant within the membrane. Following the lag time a linear steady-state appearance of drug in the receptor phase was observed over the time course of the experiments. This time course varied between 3 and 5 h depending upon the homolog.

The homologous series of compounds used in these experiments was the n-alkyl p-aminobenzoate esters. These homologous esters are either commercially available or are readily synthesized. In addition, their physicochemical properties have been characterized and described (Yalkowsky et al., 1972) and their transport properties across dimethylpolysiloxane membranes have been characterized (Flynn and Yalkowsky, 1972).

Interpretation of the transport observed in these experiments was accomplished by plotting in a linear fashion the amount of homolog appearing in the receptor phase as a fucntion of time for each homolog. The slope values of the linear steady-state portions of the above plots were divided by the area * over which the transport was occurring. These calculations resulted in steady-state flux values from saturated donor solutions for each homolog in units of mol s^{-1} cm^{-2}. A parameter such as steady-state flux obtained for a series of homologs does not allow direct comparison between homologs relative to their transport efficiencies (permeabilities) when the driving gradient for the process is the saturation solubility of each homolog, because the saturation solubilities of the homologs

* The effective area over which the diffusional process occurred was the area of exposure of the corneal membrane to the donor and receptor phases, an area defined by the diffusion apparatus.

differ. In this series of homologs the aqueous saturation solubilities decrease as the alkyl chain length is increased. In comparing the methyl homolog to the n-hexyl homolog, a decrease of greater than two orders of magnitude is observed in the aqueous saturation solubilities. Therefore, to compare the transport efficiencies of the homologs on an equimolar basis, a normalization relative to the driving gradient is achieved by dividing the experimentally determined steady-state flux values (mol s^{-1} cm^{-2}) for each homolog by the saturation solubility (mol cm^{-3}) of each homolog in the donor phase. The result of the calculation in an expression of corneal membrane permeability (cm sec^{-1}), CMP. This parameter is ideally independent of homolog concentration in the donor phase and represents the intrinsic ability of a particular homolog to undergo transport across the corneal membrane.

Since CMP is ideally independent of the driving gradient for the process, the donor phase concentration, the effect of the physicochemical properties of the permeants on the transport process can be obtained by comparing CMP to the alkyl chain length of the homologs. Fig. 1 is a plot of log CMP as a function of the number of carbon atoms in the n-alkyl chain of the homologs. A 'parabolic' relationship is seen between CMP and the n-alkyl chain length. The CMP is seen to increase from methyl to n-propyl with a maximum permeability for the n-propyl homolog. From the n-propyl through the n-hexyl homologs the permeability is seen to decrease from the maximum. This observed parabolic relationship between CMP and n-alkyl chain length is a result of change-over in a rate-limiting mass transfer step.

The transport characteristics of the n-alkyl p-aminobenzoates across a homogenous (dimethylpolysiloxane) membrane have been thoroughly described (Flynn and

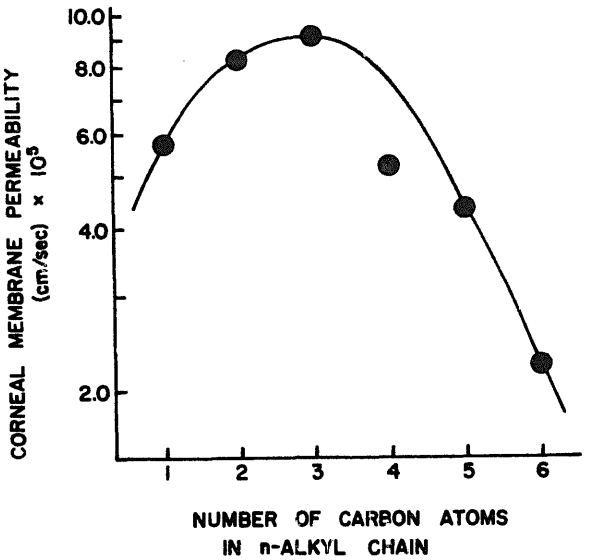

Fig. 1. A plot of log corneal membrane permeability (CMP) against the number of carbon atoms in the n-alkyl chain of the n-alkyl p-aminobenzoate esters. The CMP values were obtained by dividing the experimentally determined steady-state fluxes by the concentration of homolog in the donor phase. The experimental variability in the steady-state flux values for the 6 homologs had a range in standard deviation of 9.0–28.9%. A minimum of 6 experiments were conducted on each homolog.

Yalkowsky, 1972), wherein a change-over in the rate-liming mass transfer step was interpreted as a membrane rate-limited transfer for the lower homologs and an aqueous diffusion layer rate-limited transfer for the higher homologs. The physical model presented by Flynn and Yalkowsky (1972) for the transport of a series of homologous compounds across a homogenous membrane fully describes the parabolic profile observed in the steady-state flux from saturated donor solutions as a function of permeant structure (n-alkyl chain length or partition coefficient). The steady-state flux from a saturated donor solution represents the maximum rate of transfer for a homolog across a membrane, because the donor concentration or driving force is maximized. However, when permeabilities or permeability coefficients, which are concentration normalized steady-state fluxes determined by dividing observed steady-state fluxes by donor concentrations, are plotted as a function of permeant structure (n-alkyl chain length or partition coefficient) a parabolic behavior is not evident. This is supported by experimental data and predicted by the physical model of Flynn and Yalkowsky (1972). For a homologous series of compounds the physical model for a homogenous membrane, for lower homologs under membrane rate control, predicts permeability increases proportional to partition coefficient increases. However, the physical model for a homogenous membrane, for higher homologs under aqueous diffusion layer control, predicts a limiting permeability that is independent of membrane characteristics, partition coefficient and structural characteristics of the permeants, but dependent only upon aqueous diffusion layer thickness and diffusion characteristics of the permeants in the donor and receptor phase solvent. That is to say, a plot of log permeability as a function of n-alkyl chain length or partition coefficient will have a limiting slope of zero for higher members of a homologous series or for those homologs under aqueous diffusion layer control characterized by large partition coefficients. Therefore, a physical model based on the assumption that a membrane is homogenous cannot be used to describe the parabolic behavior of permeability as a function of structure for a series of homologous compounds.

A recent publication (Schoenwald and Ward, 1978) presented corneal membrane permeability data for a series of eleven steroids. The data is similar to that presented in Fig. 1 for the n-alkyl p-aminobenzoates, in that a parabolic relationship is demonstrated between corneal membrane permeability and steroid structure or partition coefficient. Schoenwald and Ward (1978) attempted to qualitatively resolve the parabolic dependency of corneal membrane permeability on partition coefficient in terms of a physical model, which assumes a homogenous membrane. However, a more suitable approach to resolving the mechanism of corneal drug transport is to use a physical model approach that treats independently the anatomically distinct layers of the cornea (lipid epithelial layer, aqueous stromal layer and lipid endothelial layer), rather than assuming the corneal membrane to be a homogenous membrane relative to drug transport. By this approach, lumped or overall (observed) permeability coefficients can be resolved in terms of specific permeability coefficients assigned to particular homogenous layers, such as the epithelium, stroma or endothelium, of the complex membrane. Using a physical model approach, which treats the corneal membrane as a composite or complex membrane, rather than a homogenous membrane, permits resolution of the parabolic dependency of the corneal membrane permeability on permeant structure. Mechanistic details utilizing this type of an approach will be the subject of a subsequent report.

The purpose of this communication is to report for the n-alkyl p-aminobenzoates the dependency of corneal membrane permeability in an in vitro system on the physicochemical properties or structure of permeant. It is also the intent of this communication to describe and emphasize that this parabolic permeability behavior cannot be resolved mechanistically in terms of a physical model that assumes homogeneity of the corneal membrane.

The significance of the CMP parabola is evident in drug and prodrug design for ocular use. The rate of corneal drug penetration is affected by physicochemical drug or prodrug properties. As a result, ocular drug bioavailability is affected, because of the limited contact time that a drug, which is administered by the eye drop technique, has with the absorbing corneal surface. Following the topical administration of a drug to the eye, an administered drug is removed from the absorbing corneal surface by the nasolacrimal turnover mechanism within 5 min of administration (Chrai et al., 1973; Sieg and Robinson, 1976). With the existence of a limited contact time or physiologically defined time for drug or prodrug to undergo productive corneal absorption, total corneal drug absorption can be improved by selection of a drug derivative or prodrug that possesses improved corneal membrane permeability characteristics as a function of its physicochemical properties. Actual increases in ocular bioavailability, which result from optimized permeability as a function of structural modification, can only be assessed by in vivo evaluation. However, in vitro corneal permeability studies can serve as a valuable aid in the design of drug derivatives and prodrugs for ocular use.

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