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Physicochemical factors affecting β-adrenergic antagonist permeation across cultured hamster pouch buccal epithelium

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Summary

Physicochemical factors affecting β-adrenergic antagonist permeation across an in vitro buccal epithelium model were characterized. The model consisted of primary cultures of hamster pouch buccal epithelium (CHPBE) grown on collagen-coated polycarbonate filters (3.0 μm) and placed in a side-by-side diffusion apparatus. The time, temperature, and pH dependence of the transepithelial flux of selected β-adrenergic antagonists (propranolol, alprenolol, metoprolol, pindolol, and atenolol) was followed and quantitated by fluorescence spectroscopy. Results indicate that permeation of these substances across CHPBE is dependent on, and enhanced by, higher apparent octanol/buffer distribution coefficients. The influence of increasing buffer pH was observed as an enhancement of propranolol and atenolol permeation across the tissue. In contrast, increasing buffer pH did not substantially alter the permeation of a unionizable substance, sucrose, across CHPBE. In other experiments, the temperature dependence for alprenolol and atenolol permeation across the cultured epithelium was determined. Calculated activation energies were consistent with literature values for substances crossing biomembranes by a passive mechanism. The collective findings of this study were consistent with the reported permeability and absorption characteristics of β-adrenergic antagonists observed for buccal epithelium in vivo and in other tissues. This study provides a demonstration of the utility of primary cultures of hamster buccal epithelium as a useful in vitro model for characterizing physiochemical factors affecting drug delivery by the buccal route.

Introduction

The octanol/buffer distribution coefficient and other physicochemical properties of some drugs are often used to predict the potential to diffuse across tissues or cross biological membranes. A positive relationship between distribution coefficient and permeation across a tissue has been shown, for example, with β-adrenergic antagonists (Schurmann and Turner, 1978; Schoenwald and Huang, 1983), steroids (Schoenwald and Ward, 1978), *n*-alkyl-*p*-aminobenzoate esters (Mosher and Mikkelson, 1979), and for a series of barbiturates (Huang et al., 1985). In many instances, where alternative delivery mechanisms are absent, design or development of drugs with higher octanol/buffer partition coefficient and other

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favorable physicochemical properties may be important in facilitating the delivery of drugs across some tissue barriers.

Systemic delivery of drugs administered by the buccal route is determined primarily by the physicochemical properties of the drugs (Moffat, 1971). Since buccal administration is a potentially useful route for systemic delivery of some drugs (Moffat, 1971; Squier and Johnson, 1975), development of model systems for studying drug permeation across buccal epithelium has become desirable. Recently, hamster pouch buccal epithelium (HPBE) in primary culture was developed as an in vitro model to characterize drug transport, metabolism, pharmacology, and toxicology (Tavakoli-Saberi and Audus, 1989). The purpose of this study was to characterize the primary cultures of HPBE with respect to the importance of physicochemical factors of β-adrenergic antagonists in transepithelial permeation and to demonstrate the application of the in vitro model in investigation of physical and chemical factors important in systemic drug delivery by the buccal route.

Materials and Methods

Chemicals

Propranolol hydrochloride, alprenolol tartrate, pindolol, metoprolol tartrate, atenolol and trypsin type III were purchased from Sigma Chemical Company, Saint Louis, MO. [U-^{14}C]sucrose, 350 mCi/mmol was purchased from ICN Radiochemicals, Irvine, CA. DPH (1,6-diphenyl-1,3,5-hexatriene) was obtained from Molecular Probe Eugene, OR. Plasma-derived equine serum was purchased from HyClone Laboratories, Logan, UT. Minimal Essential Medium (Eagle's modified) with Earle's Salts, glutamine and without $NaHCO_3$ were obtained from Hazelton, Lenexa, KS. Fibronectin was obtained from Boehringer Mannheim, Indianapolis, IN. Polycarbonate discs, 3 μm pore, 13 mm diameter, were purchased from Nucleopore Corporation, Pleasanton, CA. Scinti-Verse E was obtained from Fischer Company, St. Louis, MO. All other reagents were of the highest grade commercially available.

Animals

Male Syrian golden hamster (*Mesocricetus auratus*), 90–110 g, were obtained from Sasco Inc., Omaha, NE.

Isolation and culture of hamster buccal epithelial cells

Hamster pouch buccal epithelium was excised and cultured as described elsewhere (Tavakoli-Saberi and Audus, 1989). Briefly, hamsters were sacrificed by CO_2 asphyxiation and each pouch everted, excised, and washed. One longitudinal incision was made in each pouch. The pouches were then incubated in serum-free Eagle's medium containing 0.25 g/100 ml trypsin Type III for 30–45 min at 37 °C. After incubation, the pouches were washed with Eagle's medium containing 10% horse serum. The epithelial sheets were separated with forceps and shredded. Approximately 15–20 $\times 10^4$ cells/cm^2 were seeded in 100 mm or 60 mm plastic culture dishes containing polycarbonate discs coated with cross-linked rat-tail collagen and fibronectin. The cells were incubated at 95% humidity, 5% CO_2 at 37 °C in a culture medium consisting of Dulbecco's modified Eagle's medium (MEM) containing 10% plasma-derived horse serum, 100 μg/ml penicillin G, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B, 300 μg/ml ascorbic acid, 2.2 g/l sodium bicarbonate, 20 mM HEPES, pH 7.4.

Drug assay

The fluorescence of β-adrenergic antagonist drug samples was measured with excitation and emission wavelengths shown in Table 1. Fluorescence measurements were made with an SLM-4800

TABLE 1

Fluorescence emission and excitation wavelengths for β-adrenergic antagonists

Compound	Excitation (nm)	Emission (nm)
Propranolol	295	340
Alprenolol	270	300
Metoprolol	272	307
Pindolol	250	360
Atenolol	270	305

spectrofluorometer (SLM-Aminco, Urbana, IL). [U-^{14}C]Sucrose was assayed for ^{14}C with a Beckman 6800 scintillation counter (Beckman Instruments, Inc., Fullerton, CA).

Buffer solutions

PBSA buffer (129 mM NaCl, 2.5 mM KCl, 7.4 mM Na_2HPO_4, 1.3 mM KH_2PO_4, 0.63 mM $CaCl_2$, 0.74 mM $MgSO_4$, 5.3 mM glucose, pH 7.4, 8.5, 9, 9.45) and epithelial assay buffer (122 mM NaCl, 25 mM $NaHCO_3$, 10 mM D-glucose, 3 mM KCl, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4, 1.4 mM $CaCl_2$, 10 mM HEPES, pH 6.8, 7.4 and 8.2 were prepared and pH values adjusted at room temperature.

Determination of distribution coefficients

PBSA buffer, pH = 7.4, and octanol were mutually saturated at 37°C by shaking overnight, centrifuged at 1000 *g* for 5 min, and left to separate for 1 h. The distribution coefficient at 37°C was determined by dissolving drug in the aqueous buffer phase and shaking with octanol at 37°C for 1 h. The mixture was centrifuged at 1000 *g* for 5 min and then left to separate for 1 h at 37°C. The volumes of each phase were chosen so that the drug concentration in the aqueous phase, before and after extraction, could be measured by fluorometry. The distribution coefficient (DC) was calculated by method of Schoenwald and Huang (1983).

Diffusion studies

Horizontal Side-Bi-Side diffusion cells (Crown Glass Company, Inc., Somerville, NJ) were used for transport studies as previously described (Tavakoli-Saberi and Audus, 1989). Briefly, collagen-coated polycarbonate disks with or without cells were placed in the diffusion cells such that the collagen matrix or epithelial cells always faced the donor chamber of the diffusion cell. The diameter of the diffusion area was 9 mm. The water jacket surrounding the sample chambers was thermostated at the desired temperature with a circulating water bath. The volume of the diffusion cell sample chamber was maintained at 3.0 ml of buffer during experiments and continually stirred with magnetic stir bars.

The donor chamber was pulsed with different β-blocking agents and a 0.1 ml aliquot of sample removed from the receptor chamber at various times for analysis by fluorometry or scintillation spectrometry. An aliquot (0.1 ml) of fresh assay buffer was added back to the receptor chamber after each withdrawal to maintain constant volumes.

Apparent permeability coefficients were estimated as previously described (Tavakoli-Saberi and Audus, 1989).

Activation energies were calculated from the Arrhenius equation:

$$\log k_p = \log A - E/2.3RT$$

In which k_p is the permeability coefficient, A is the frequency factor, E the activation energy, R the gas constant and T the absolute temperature. When $\log k_p$ is plotted against $1/T$, a straight line is obtained, the slope of which is proportional to the activation energy (Blank et al., 1967). The fraction of unionized drug at a given pH was calculated from Henderson-Hasselbalch equation:

$$fu = 1/(1 + 10^{(pK_a - pH)})$$

(Schurmann and Turner, 1978). The apparent permeability coefficient for the cells (P_{cells}) was calculated from the following relationship: $1/P_{pc+cells} = 1/P_{cells} + 1/P_{pc}$ (Stein, 1986).

Cell membrane labeling procedure

Four-day old cultured hamster epithelial cells were homogenized as described elsewhere (Tavakoli-Saberi and Audus, 1989) and centrifuged at 1000 *g*. The pellets were collected, washed with PBS, centrifuged 5 minutes at 1000 *g* and labeled with diphenyl hexatriene (DPH) by adding 2.5 μl of a 1 mM freshly prepared stock of DPH in tetrahydrofuran to a 2.5 ml of membrane suspension (corresponding to about 1×10^6 cells/ml) and kept in the darkness 30 min before the fluorescence measurements were made. The fluorescence anisotropy of DPH-labeled membrane was measured according to Audus et al. (1988).

Results and Discussion

The permeability of cultured hamster pouch buccal epithelium (CHPBE) to 5 different β-adrenergic antagonists was studied. These compounds were all similar with respect to molecular weight and pK_a and differed in their lipophilicity, as distinguished by apparent octanol/buffer distribution coefficients determined here (Table 2). Apparent permeability coefficients were calculated from the time-dependent flux of the β-adrenergic antagonists across collagen-coated polycarbonate discs alone and in the presence of CHPBE (Fig. 1A,B). The calculated apparent permeability coefficients for the β-adrenergic antagonists' permeation across CHPBE were dependent on the apparent distribution coefficients of the substance (Fig. 2). In general, higher apparent permeability coefficients were observed with the more lipophilic compounds. However, the relationship between relative lipophilicity and permeability was non-linear. For those compounds with higher octanol/buffer distribution coefficients, e.g., alprenolol and propranolol, permeation across the tissue may be by limited cellular retention of the drugs, thus accounting for the observed non-linearity. By other fluorescence spectroscopic methods, we have observed reversible uptake of only the more lipophilic adrenergic antagonists into buccal epithelial cells (Tavakoli-Saberi and Audus, unpublished observations). An alternative explanation for the sigmoidal relationship between lipophilicity and permeability, the presence of a significant hydrophilic barrier in the system, such as the stroma behind corneal epithelium (Schoenwald and Huang, 1983), does not seem likely. A similar sigmoidal relationship between β-blocker permeability and distribution coefficient has also been observed for a monolayer of endothelial cells grown on polycarbonate filters (Van Bree et al., 1988). In that system, as proposed here, the permeability of the more lipophilic compounds was attributed to cellular retention of the more lipophilic β-adrenergic antagonists.

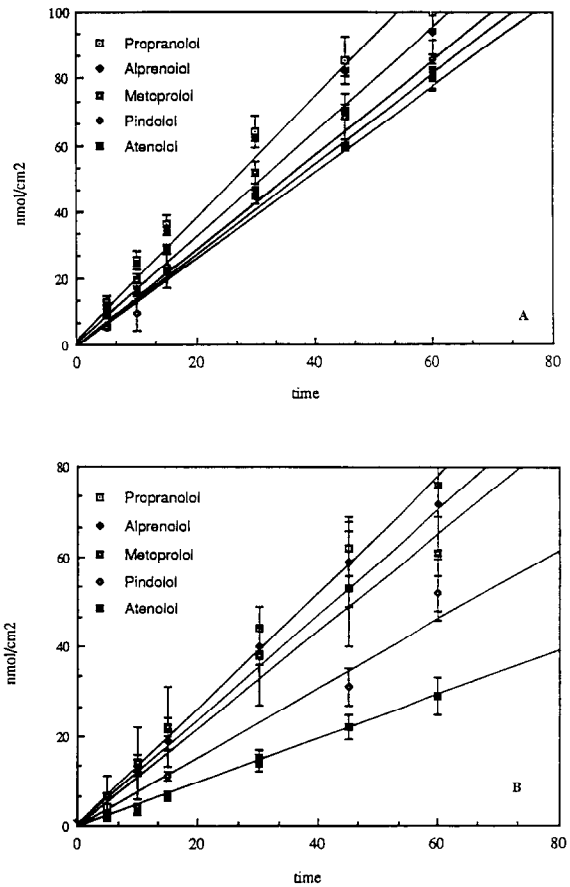

Fig. 1. Time dependence of the permeation of β-adrenergic antagonists (100 μM) across collagen-coated polycarbonate discs without (A) and with (B) primary cultures of hamster pouch buccal epithelium present. Each data point represents the mean ± S.D. for 3 separate experiments.

TABLE 2

Molecular weights, ionization constants (pK_a) and distribution coefficients for selected β-adrenergic antagonists

Compound	MW	pK_a *	log DC
Propranolol	259.34	9.45	1.540
Alprenolol	249.34	9.70	1.230
Metoprolol	267.38	9.70	0.068
Pindolol	248.30	8.80	−0.200
Atenolol	266.30	9.55	−1.397

* Hinderling et al., 1984.

The pH-dependence of the flux of propranolol, and atenolol (at pH 6.8 and 8.2) across CHPBE suggests that the diffusion of these drugs across the tissue depends on the ionization state of the molecule (Fig. 3A,B). A plot of apparent permea-

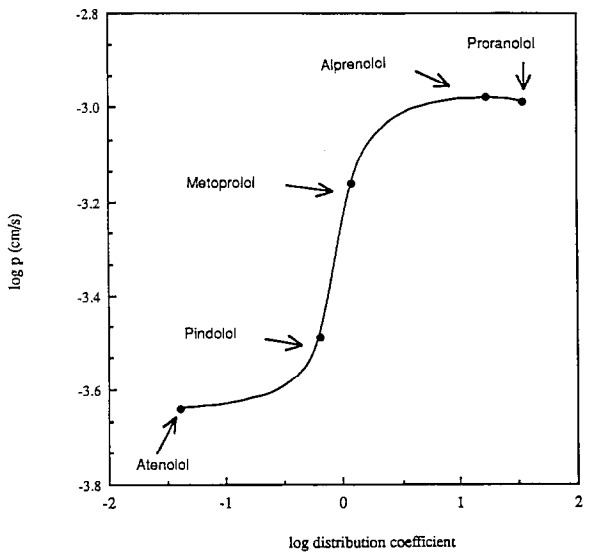

Fig. 2. Relationship between apparent permeability coefficient and octanol/buffer distribution coefficient for β-adrenergic antagonist permeation across primary cultures of hamster pouch buccal epithelium.

bility coefficient versus the unionized fraction of propranolol at various pHs also clearly shows a non-linear relationship (Fig. 4). When the fraction of unionized drug was essentially zero (at pH = 6.8), permeation of propranolol across CHPBE was substantially attenuated. A small increase in the unionized fraction (fu) yielded a large increase in the permeability coefficient (p) of propranolol (e.g., for $fu = 0.05$, $p \cong 2.5 \times 10^{-4}$ cm/s). These findings suggest that the fraction of propranolol unionized was important for the transport across the cultured buccal epithelium. Permeation across CHPBE by sucrose, a biomembrane impermeant marker, at selected pHs 6.8, 7.4 and 8.2, was consistent (5.76 ± 1.42% of the donor concentration) and thus pH-independent, implying that epithelial cells were not damaged over the pH range examined. Our findings were consistent with Schurmann and Turner (1978) who similarly demonstrated the absence of significantly greater buccal absorption of propranolol at higher pHs in vivo.

Alprenolol and atenolol were chosen to examine the effect of temperature on β-adrenergic antagonist permeation across buccal epithelium at pH 7.4. The flux of both alprenolol (Fig. 5) and atenolol (not shown) across CHPBE was temperature-dependent with maximal permeation across the tissue occurring at higher temperatures. The apparent permeability coefficients for both drugs were calculated at various temperatures and the activation energies calculated from the slope of plot of log apparent permeability coefficient versus

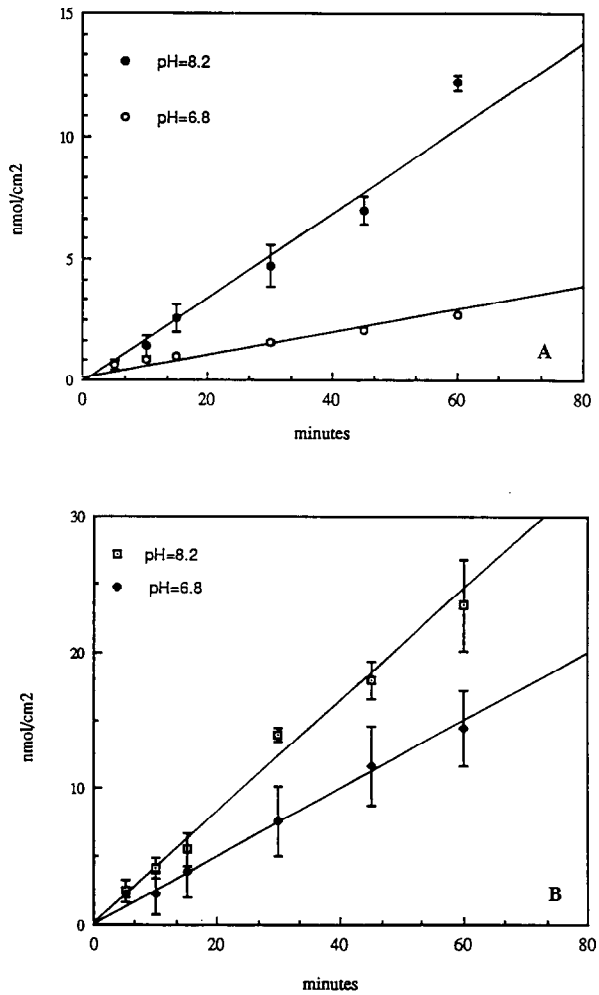

Fig. 3. pH dependence of the permeation of β-adrenergic antagonists across primary cultures of hamster pouch buccal epithelium. A: propranolol (10 μM). B: atenolol (100 μM). Each data point represents the mean ± S.D. for 3 separate experiments.

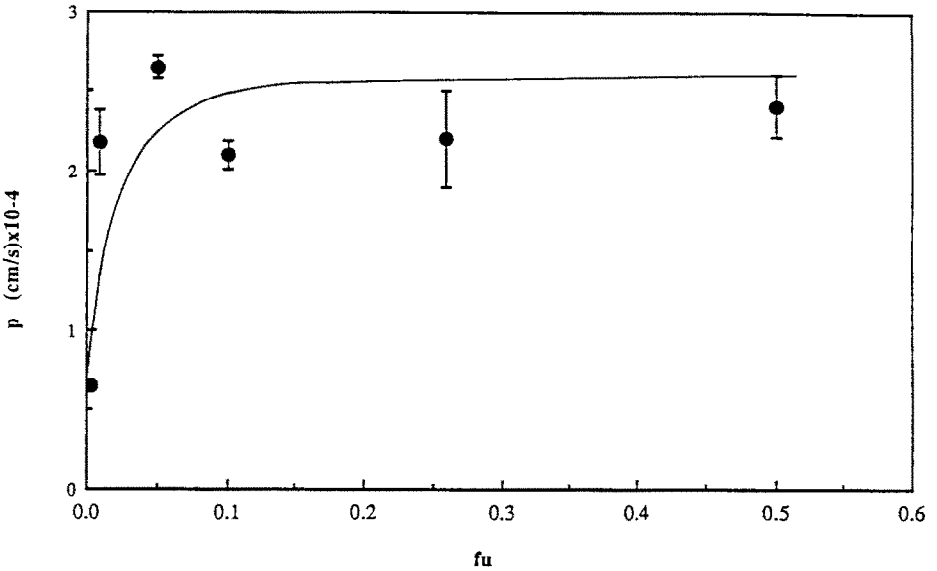

Fig. 4. Relationship between apparent permeability coefficient and unionized fraction (*fu*) for permeation of propranolol across primary cultures of hamster pouch buccal epithelium. Each data point represents the mean ± S.D. for 3 separate experiments.

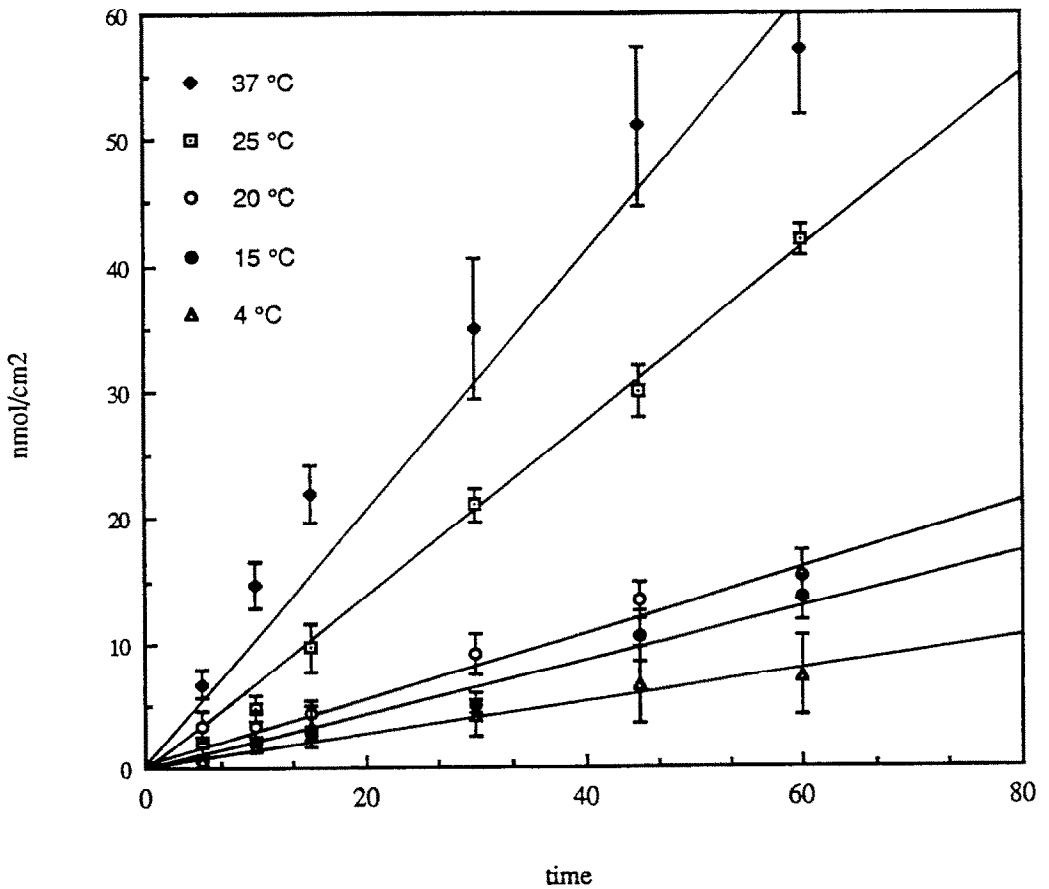

Fig. 5. Temperature-dependence of alprenolol (100 μM) permeation across primary cultures of hamster pouch buccal epithelium. Each data point represents the mean ± S.D. for 3 separate experiments.

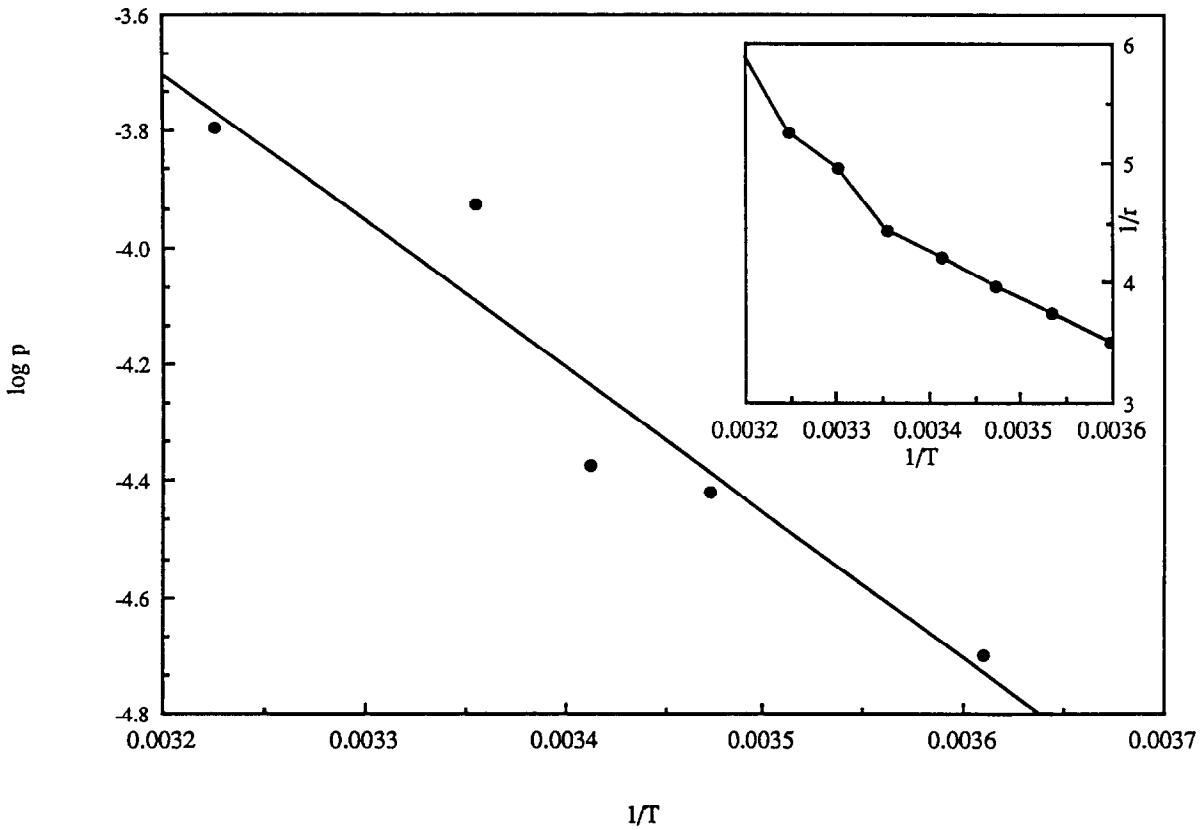

Fig. 6. Arrhenius plot generated for the temperature-dependent permeation of alprenolol across primary cultures of hamster pouch buccal epithelium. Inset: effect of temperature on the fluorescence anisotropy of diphenyl-1,3,5-hexatriene labeled buccal epithelial cells (i.e., the phase-transition of the membrane lipids). Each point is mean of 3 separate measurements for both experiments.

reciprocal of temperature (alprenolol, Fig. 6). The activation energies for alprenolol and atenolol were 33.4 ± 5.06 and 37.14 ± 0.33 kJ/mol, respectively. The action energies calculated here for alprenolol and atenolol were consistent with activation energies for the passive diffusion of substances across biomembranes (Stein, 1986). The relationship between apparent permeability coefficient and reciprocal temperature parallels the temperature-dependent phase transition of buccal cell membranes labeled with the hydrophobic membrane probe, DPH (inset, Fig. 6) (Shinitzky and Barenholtz, 1978). The apparent correlation between membrane lipid phase transition and membrane permeability shown here would also suggest that the state of buccal cell membrane lipids also plays a role in regulating the permeation of β-adrenergic antagonists across buccal epithelium.

Drug delivery by the buccal route offers certain advantages including convenience, rapid absorption for some drugs, and avoidance of first pass metabolism. However, in the context of buccal drug delivery, disadvantages also exist and among them is the absence of specific carrier-mediated transepithelial transport mechanisms that one might manipulate or employ in drug delivery schemes (Squier and Johnson, 1975). Development and design of drugs destined for delivery by the buccal route will more likely be based on physiochemical properties favoring efficient permeation across the epithelial barrier. Tissue culture systems offer potentially important alternative experimental tools in drug delivery studies both economically and for humane considerations, as initial screening models for drug transport, metabolism, pharmacology, and toxicology studies (Handler,

1983; Prunieras and Delescluse, 1984; Klausner, 1987). Results from the present study demonstrate the utility and sensitivity of the cultured buccal epithelium as an in vitro model for studying physicochemical factors important for drug delivery by buccal route.

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References

Ahmed, I., Gokhale, R.D., Shah, M.V. and Patton, T.F., Physicochemical determinants of drug diffusion across the conjunctiva, sclera and cornea. *J. Pharm. Sci.*, 76 (1987) 583–586.

Audus, K.L., Shinogle, J.A., Guillot, F.L. and Holthaus, S.R., Aluminium effects on brain microvessel endothelial cell monolayer permeability. *Int. J. Pharm.*, 45 (1988) 249–257.

Blank, I.H., Scheuplein, R.J. and McFarlane, D.J., Mechanism of percutaneous absorption. III. The effect of temperature on the transport of non-electrolytes across the skin. *J. Invest. Dermatol.*, 49 (1967) 582–589.

Handler, J.S., Use of cultured epithelia to study transport and its regulation. *J. Exp. Biol.*, 106 (1983) 55–66.

Hinderling, P.H., Schmidlin, O. and Seydel, J.K., Quantitative relationships between structure and pharmacokinetics of β-adrenoceptor blocking agents in man. *J. Pharmacokin. Biopharm.*, 12 (1984) 263–287.

Huang, H.H., Kimura, R., Nassar, R.B. and Hussain, A., Mechanism of nasal absorption of drugs I: physicochemical parameters influencing the rate of in situ nasal absorption of drugs in rats. *J. Pharm. Sci.*, 74 (1985) 608–611.

Klausner, A., Tissue culture for improved toxicology. *Biotechnology*, 5 (1987) 779–785.

Moffat, A.C., Absorption of drugs through oral mucosa. *Top. Med. Chem.*, 4 (1971) 1–27.

Mosher, G.L. and Mikkelson, T.J., Permeability of the *n*-alkyl-*p*-aminobenzoate esters across the isolated corneal membrane of the rabbit. *Int. J. Pharm.*, 2 (1979) 239–243.

Prunieras, M. and Delescluse, C., Epidermal culture systems in skin pharmacology. *Br. J. Dermatol.*, III, Suppl. 27 (1984) 43–57.

Schoenwald, R.D. and Huang, H.S., Corneal penetration behavior of β-blocking agents I: Physicochemical factors. *J. Pharm. Sci.*, 72 (1983) 1266–1272.

Schoenwald, R.D. and Ward, R.L., Relationship between steroid permeability across excised rabbit cornea and octanol-water partition coefficients. *J. Pharm. Sci.*, 67 (1978) 786–788.

Schurmann, W. and Turner, P., A membrane model of the human oral mucosa as derived from buccal absorption performance and physico-chemical properties of β-blocking drugs atenolol and propranolol. *J. Pharm. Pharmacol.*, 30 (1978) 137–147.

Shinitzky, M. and Barenholtz, Y., Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim. Biophys. Acta*, 515 (1978) 367–394.

Squier, C.A. and Johnson, N.W., Permeability of oral mucosa. *Br. Med. Bull.*, 31 (1975) 169–175.

Stein, W.D., *Transport and Diffusion Across Cell Membranes*, Academic Press, Orlando, FL, 1986.

Tavakoli-Saberi, M.R. and Audus, K.L., Cultured buccal epithelium: an in vitro model derived from hamster pouch for studying transport and metabolism. *Pharm. Res.*, 6 (1989) 160–166.

Van Bree, J.B.M.M., De Boer, A.G., Danhof, M., Ginsel, L.A. and Breimer, D.D., Characterization of in vitro blood brain barrier: effect of molecular size and lipophilicity on cerebrovascular endothelial transport rates of drugs. *J. Pharmacol. Exp. Ther.*, 24 (1988) 1233–1239.