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WATER PERMEABILITY OF ACINAR CELL MEMBRANES IN THE ISOLATED PERFUSED RABBIT MANDIBULAR SALIVARY GLAND

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SUMMARY

1. The diffusive water permeability of epithelial cell membranes in the perfused rabbit mandibular salivary gland was measured at 37 °C by a ^1H nuclear magnetic resonance relaxation method using an extracellular relaxation reagent, gadolinium diethylenetriaminepentaacetic acid (Gd(DTPA)).

2. In glands perfused with a HEPES-buffered solution containing 10 mmol l^{-1} Gd(DTPA), the spin-lattice (T_1) relaxation of the water protons showed two exponential components. The water compartment responsible for the slower component corresponded in magnitude to $71 \pm 5\%$ of the wet weight of the gland, and was attributed to the exchangeable intracellular water of the acinar cells.

3. The rate constant for water efflux from the cells was estimated to be $4{\cdot}1 \pm 0{\cdot}1$ s^{-1} which would be consistent with a diffusive membrane permeability (P_d) of approximately 3×10^{-3} cm s^{-1}. Stimulation with acetylcholine (10^{-6} mol l^{-1}) did not cause any detectable change in membrane water permeability.

4. Since the basolateral membrane probably provides the main pathway for water efflux, the osmotic water permeability of this barrier (expressed per gland) was estimated to be less than 6·2 cm^3 s^{-1}. This would be insufficient to account for the generation of a near-isosmotic fluid at the flow rates observed during secretion, and suggests that a substantial fraction of the flow of water occurs via a paracellular route.

INTRODUCTION

When stimulated with muscarinic agonists, the isolated, perfused rabbit mandibular gland can secrete a volume of fluid equivalent to its own mass in less than 2 min (Case, Conigrave, Novak & Young, 1980). Although the ion transport mechanisms responsible for driving this rapid flow of water are now quite well characterized (reviewed by Cook & Young, 1989), the route of water flow across the secretory epithelium remains uncertain. In a previous study (Case, Cook, Hunter, Steward & Young, 1985; Howorth, Case & Steward, 1987), in which the permeability of the gland to small polar non-electrolytes was examined, evidence of solvent drag

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enabled us to estimate the equivalent pore radius of the water pathway to be 0·4 nm. We were unable, however, to establish whether water flow was via a transcellular or paracellular route.

The purpose of the present study was to measure the water permeability of the epithelial cell membranes and thus to estimate the size of the osmotic gradient that would be required to drive water through the transcellular pathway at the rates observed during secretion. Since intact exocrine glands are inaccessible to the optical methods that have been used to measure the osmotic water permeability of some other epithelia (e.g. Persson & Spring, 1982), we have used a nuclear magnetic resonance (NMR) method that measures the closely related, diffusive water permeability (Fabry & Eisenstadt, 1975).

The principle of the method is as follows. When hydrogen nuclei (protons) are placed in a strong, uniform magnetic field, they show a small net magnetization along the direction of the field. A short pulse of radio frequency (RF) radiation is used to invert this net magnetization, and the rate of recovery to the normal orientation, described by the spin-lattice relaxation time T_1, is measured. Most of the proton NMR signal from biological tissue derives from water, and it is possible to distinguish between intracellular and extracellular water molecules by adding an impermeant relaxation reagent to the extracellular fluid. This has the effect of accelerating the relaxation of the extracellular water protons by two or three orders of magnitude. In the absence of diffusive water exchange across the plasma membrane, the intracellular water protons would continue to relax at the slower rate. If the water permeability of the membrane is sufficient, however, the intracellular water protons can relax more rapidly by diffusing out the cell and into the presence of the relaxation reagent. If this is the case, the rate constant of diffusive water efflux can be estimated from the relaxation rate of the intracellular water protons.

In a previous study, values obtained by this method for the water permeability of *Necturus* gallbladder epithelial membranes (Steward & Garson, 1985) were in good agreement with those based on optical measurements of the changes in cell volume induced by osmotic gradients (Persson & Spring, 1982). Although the NMR method measures diffusive rather than osmotic water permeability, and the two parameters are not necessarily equal (Finkelstein, 1987), the method has the advantage that it is not subject to the unstirred-layer artifacts associated with osmotic flow measurements (Barry & Diamond, 1984).

Most previous measurements of water permeability by NMR have used paramagnetic Mn^{2+} ions as the extracellular relaxation reagent. Since it is known that millimolar concentrations of free Mn^{2+} ions inhibit salivary secretion in response to muscarinic stimulation (Young, Cook, Evans & Pirani, 1987), probably through their effects on cellular Ca^{2+} homeostasis, we have used instead the stable, paramagnetic lanthanide complex, gadolinium diethylenetriaminepentaacetic acid (Gd(DTPA)).

Some of the data presented here have previously been communicated to the Physiological Society (Case, Rawlings, Seo & Steward, 1989).

METHODS

Gland perfusion

Female half-lop rabbits weighing 2·0–2·5 kg were anaesthetized by intraperitoneal injection of urethane (1·5 g kg^{-1} body weight). Mandibular glands (wet weight *ca* 0·4 g) were isolated and cannulated for vascular perfusion (Case *et al.* 1980). To minimize the development of oedema, the glands were perfused, using a peristaltic pump (Watson Marlow, Falmouth), at 0·5 ml min^{-1}, one-eighth of the rate used in previous studies (Seo, Steward, Mackenzie & Case, 1988). The glands were thus expected to be mildly hypoxic, and, indeed, ^{31}P NMR spectroscopy confirmed that the glands contained slightly more inorganic phosphate and slightly less ATP than glands perfused at 4·0 ml min^{-1}. However, the content of inorganic phosphate and ATP stabilized quickly and remained constant over a period of several hours indicating that there was no progressive decline in the metabolic status of the preparation.

The relaxation reagent Gd(DTPA) was added to the perfusate to accelerate the relaxation of the extracellular water in the gland. The complex was prepared *in situ* (Pike, Marmush, Balschi, Lenkinski & Springer, 1983) from $GdCl_3$ (Johnson Matthey Chemicals) and DTPA (Sigma, USA). The composition of the perfusate containing Gd(DTPA) was (in mmol l^{-1}): Na^+ 144, K^+ 4·5, Cl^- 132, Ca^{2+} 1·5, Mg^{2+} 1, SO_4^{2-} 1, HEPES (2-(4-hydroxyethyl)-1-piperazine-ethanesulphonic acid) 10, D-glucose 5 and Gd(DTPA) 10. When Gd(DTPA) was omitted from the perfusate, it was replaced isosmotically by 15 mmol l^{-1} NaCl. In a few experiments Mn(EDTA) was used as the relaxation reagent. The composition of the perfusate containing Mn(EDTA) was (in mmol l^{-1}): Na^+ 144, K^+ 4·5, Cl^- 144, Ca^{2+} 1·5, Mg^{2+} 1, SO_4^{2-} 1, HEPES 10, D-glucose 5 and Mn(EDTA) 15. The solutions were all gassed with 100% O_2 and pH was adjusted to 7·4.

The perfused gland was suspended in a glass tube (18 mm internal diameter) and maintained at 37 °C. The effluent perfusate was aspirated from the bottom of the tube to prevent it from contributing to the NMR signal. The signal originating from water in the tubing contributed less than 2% of the total water signal obtained from the preparation.

NMR measurements

^1H NMR signals were collected at 200·4 MHz on a BIOSPEC 150/4·7 spectrometer (Oxford Research Systems, Coventry) equipped with a horizontal-bore 4·7 T magnet. The coil of the home-built ^1H probe was a 25 mm diameter, two-turn solenoid of 5·5 mm copper ribbon. The time course of spin-lattice (T_1) relaxation of water protons in the perfused gland was measured by an inversion–recovery pulse sequence: D–180 deg–τ–90 deg–acquire (Farrar & Becker, 1971). In this method, an initial relaxation delay (D) allows the protons in the gland to reach equilibrium and then a 180 deg RF pulse is used to invert their net magnetization. This is followed by a recovery period (τ) during which the protons undergo relaxation. The magnetization remaining after a given recovery period is then sampled by applying a 90 deg RF pulse. This causes the protons to generate a detectable signal (free induction decay, FID) whose initial amplitude is determined by the magnitude and polarity of the net magnetization. The time course of relaxation is thus obtained by measuring the initial amplitude of the FID over a range of recovery times.

In these experiments, the recovery time (τ) was varied from 0·01 to 0·45 s and also set to 5 s for estimation of the equilibrium magnetization. The relaxation delay (D) was normally 5 s but was increased to 15 s for glands perfused without the relaxation reagent. For each set of measurements, the 180 deg pulse width was determined accurately (usually *ca* 60 μs), and the 90 deg pulse width was set to one-half of this value. The spectral width was 50 kHz and the data block size was 2048 points. Four scans with alternating phase were used. Approximately 10 min were required to complete a full set of relaxation measurements.

Data analysis

The analysis of these data has been described previously (Fabry & Eisenstadt, 1975, 1978) and is based on a simple two-site exchange model (Woessner, 1961). We use here the same notation as Steward & Garson (1985). Briefly, from sets of relaxation data which showed two exponential components, the following parameters were determined by least-squares curve fitting: (i) the rate constant of the faster component (ϕ_A), (ii) the rate constant of the slower component (ϕ_B) and (ii) the zero-time intercept (H) of the slower component. Also measured as an index of the total water content of the gland was (iv) the initial intensity (M) of the FID following a 90 deg RF pulse.

Assuming that the time course of the slow component reflected water efflux from the cells (see Discussion), the following derived parameters (illustrated in Fig. 1) were calculated: (v) the rate constant of diffusive water efflux from the cells given by

$$k_x = \phi_B - k_i, \tag{1}$$

where k_i is the rate constant for the relaxation of intracellular water; (vi) the intracellular water content (N) expressed as a fraction (f_i) of the total water content

$$f_i = N_i/(N_i+N_o) = H(\phi_A-\phi_B)/(\phi_A+k_x-k_i); \tag{2}$$

(vii) the rate constant of diffusive water influx into the cells:

$$k_y = k_x N_i/N_o = k_x f_i/(1-f_i), \tag{3}$$

and (viii) the intracellular water content (in g)

$$N_i = f_i CM, \tag{4}$$

where C is an instrumental calibration constant (see below).

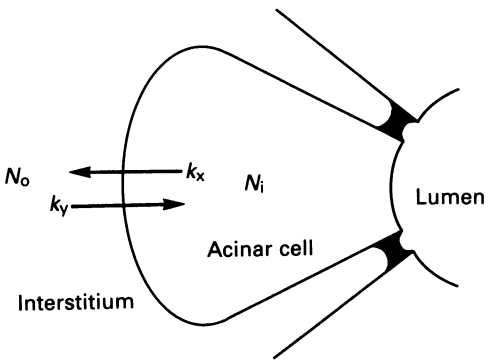

Fig. 1. Two-site model for water exchange across the basolateral membrane of the salivary acinar cell. The kinetics of exchange are described by the rate constants of water efflux and influx, k_x and k_y, and by the water contents of the intracellular and extracellular compartments, N_i and N_o.

The rate constant for the relaxation of intracellular water, k_i, was assumed to be 1·0 s^{-1}, a value typical of most mammalian cells (Mathur-De Vré, 1979) although the results of the calculations were relatively insensitive to the precise value assigned to this parameter.

The calibration constant C used to calculate absolute water content was determined from the correlation between the initial intensity (M) of the free induction decay and the measured total wet weight of the gland. Under the conditions of these experiments the value of C was 0·121.

All numerical values are expressed as the mean and standard error of the mean (mean ± S.E.M.). For tests of statistical significance, Student's t test was used, and P values less than 0·05 were accepted as indicating significance.

RESULTS

Relaxation of water in perfusates containing relaxation reagents

The spin-lattice (T_1) relaxation time course of samples of perfusate containing various concentrations of Gd(DTPA) or Mn(EDTA) all showed single-component exponential decays (results for 10 mmol l^{-1} Gd(DTPA) are shown in Fig. 2*A*). The rate constant of relaxation ($\phi = 1/T_1$) was found to be linearly related to the concentration of the relaxation reagent. The rate constants of solutions containing Gd(DTPA) were approximately 50% greater than those of solutions containing the same concentration of Mn(EDTA). Both 10 mmol l^{-1} Gd(DTPA) and 15 mmol l^{-1} Mn(EDTA) accelerated the relaxation of water protons by a factor of approximately

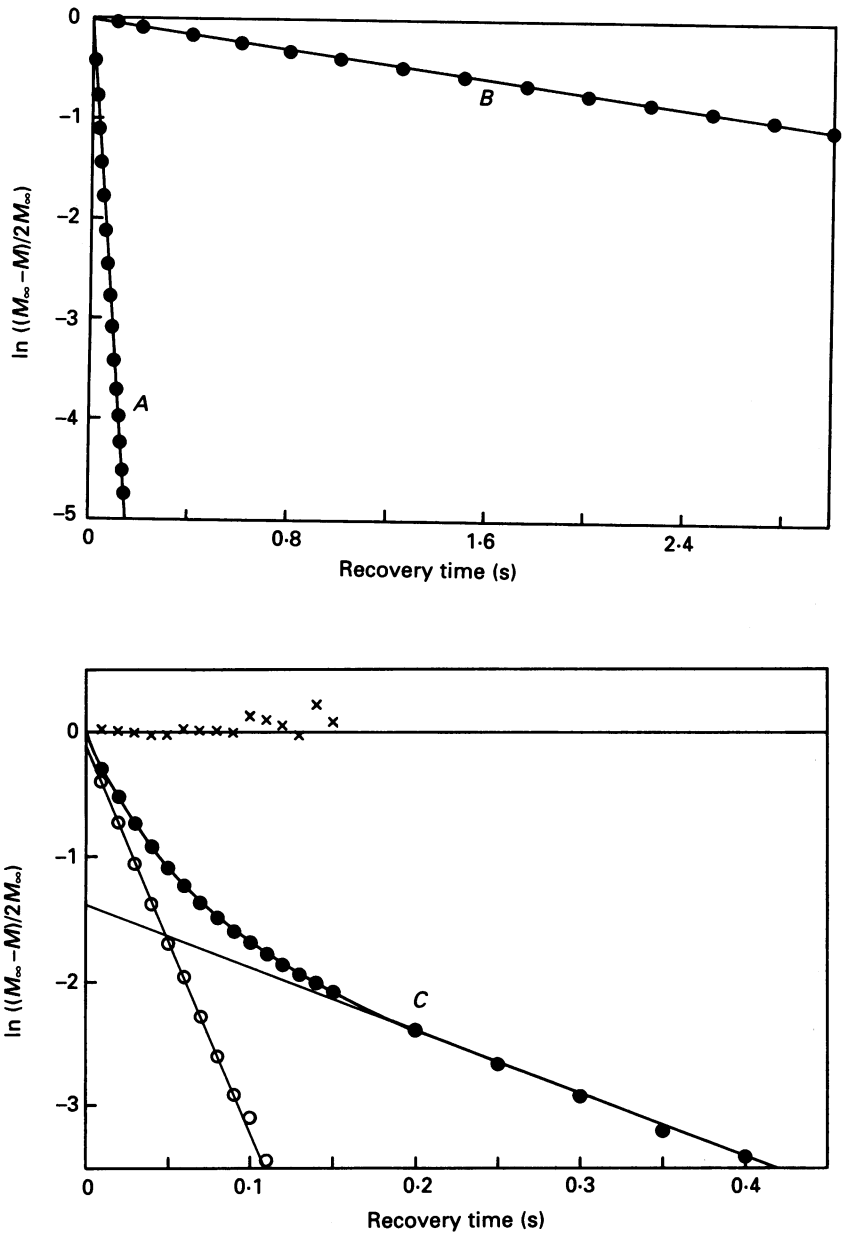

Fig. 2. Spin-lattice relaxation of water protons in a sample of perfusate (*A*) and in the perfused rabbit mandibular gland at 37 °C (*B* and *C*). Data are plotted as the natural logarithm of the difference of the magnetization (*M*), after the recovery time, from its equilibrium value (M_∞). *A*, a sample of perfusate containing 10 mmol l^{-1} Gd(DTPA). The rate constant (ϕ) obtained by linear regression was $31{\cdot}3 \pm 1{\cdot}1$ s^{-1}. *B*, a single gland perfused without relaxation reagent ($\phi = 0{\cdot}35 \pm 0{\cdot}01$ s^{-1}). *C*, a single gland perfused with 10 mmol l^{-1} Gd(DTPA) (note the different time scale from *A* and *B*). The rate constant of the slow component (ϕ_B) was $5{\cdot}1 \pm 0{\cdot}5$ s^{-1} and the zero-time intercept (*H*) was 27·4 %. Also shown in *C* is the fast component of relaxation obtained by subtraction of the fitted slow component from the original data (○, $\phi_A = 31{\cdot}1 \pm 0{\cdot}9$ s^{-1}), and the residuals of the regression analysis (×). The values of the rate constants given above are the regression coefficients and their 95 % confidence limits.

100 ($\phi = ca$ 30 s^{-1}). The relaxation reagents also slightly shifted the resonance frequency of water, although the shifts were negligible at the concentrations used in this study.

Relaxation of water in the perfused rabbit mandibular gland

In the absence of relaxation reagent, the relaxation of water protons in the salivary gland showed a single, slow exponential (Fig 2*B*). This was to be expected

TABLE 1. Measured relaxation parameters (ϕ_A, ϕ_B and H) and calculated values of the rate constants of diffusive exchange (k_x and k_y) and the content of exchangeable intracellular water (f_i and N_i)

	ϕ_A (s^{-1})	ϕ_B (s^{-1})	H (%)	k_x (s^{-1})	k_y (s^{-1})	f_i (%)	N_i (g)	n
Control	32·7±0·6	5·1±0·1	18·6±1·0	4·1±0·1	0·69±0·04	14·3±0·7	0·30±0·01	16
ACh (10^{-6} mol l^{-1})*	30·7±1·1	4·9±0·2	20·2±1·3	3·9±0·2	0·71±0·06	15·4±0·9	0·30±0·01	6
Recovery†	29·4±1·1	4·8±0·3	19·6±1·2	3·1±0·2	0·67±0·07	14·8±0·7	0·27±0·01	5

* Data collected 20–30 min after the onset of stimulation with acetylcholine (ACh).
† Data collected 25–35 min after a 30 min period of stimulation with ACh.
Values are means±S.E.M. from n experiments.

since the intrinsic relaxation rates of the intracellular and extracellular water would be of the same order of magnitude, and probably significantly smaller than the rate constants of diffusive water exchange across the membrane. The single rate constant for relaxation that was obtained (0·35 s^{-1}) would therefore represent a weighted average of the intrinsic intracellular and extracellular relaxation rates.

Relaxation of water in glands perfused with relaxation reagents

When 10 mmol l^{-1} Gd(DTPA) was added to the perfusate, the relaxation time course of the perfused gland showed two clearly resolved components (Fig. 2*C*). Values of the rate constants of the fast and slow components (ϕ_A and ϕ_B), and the zero-time intercept of the slow component (H) were determined by least-squares curve fitting and are listed in Table 1. From these, the rate constants of water efflux and influx across the plasma membrane (k_x and k_y) were calculated using eqns (1) and (3) (see Methods). The fractional and absolute values of the exchangeable intracellular water content (f_i and N_i) were obtained from eqns (2) and (4), and are given in Table 1.

This analysis assumes that there is a simple, diffusive exchange of water between the intracellular and extracellular compartments, where the relaxation rates of the water protons differ substantially. This interpretation can be justified as follows. The rate constant of the fast component of relaxation (32·7±0·6 s^{-1}, $n = 16$) was very similar to that of the perfusate alone (ca 30 s^{-1}) suggesting that it derived from the extracellular water of the perfused gland. As would be expected, if there were measurable water exchange across the membrane, the rate constant of the slow component (5·1±0·1 s^{-1}) was significantly greater than the estimated intracellular relaxation rate in the absence of Gd(DTPA) (ca 1 s^{-1}).

The relationship between the size of the water compartment responsible for the

slow component (N_i) and the wet weight of the gland was determined using data from eleven unstimulated glands. Immediately after the accumulation of the relaxation data, the oedematous connective tissue of each gland was removed by dissection and the wet weight (W) of the remaining cellular tissue measured. The ratio N_i/W was

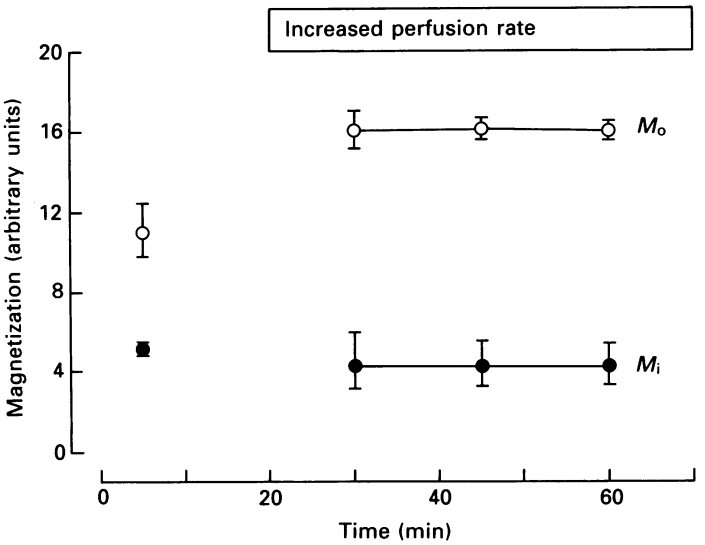

Fig. 3. Changes in the initial magnetization due to the slow (M_i, ●) and the fast (M_o, ○) components of relaxation when the perfusion rate was increased from 0·05 to 4 ml min^{-1}. Values were obtained by least-squares regression analysis of full sets of relaxation data accumulated over 10 min periods from a single gland. Error bars show the 95% confidence limits of the regression coefficients.

$0{\cdot}71 \pm 0{\cdot}05$ ($n = 11$), indicating that the slow component was derived from a water population comparable in size with that of the cellular tissue mass. Since acinar cells constitute by far the largest part of the total cell population of the gland (73% of the total parenchymal volume in the rat mandibular gland; Tamarin & Sreebny, 1965), it seems reasonable to attribute most of the slowly relaxing water signal to the intracellular water of the acinar cells.

Effects of perfusion rate and perfusate osmolarity

To assess the validity of this interpretation further, three additional experiments were performed. First, the extracellular volume of the preparation was expanded by increasing the perfusion rate from 0·5 to 4 ml min^{-1}. Changes in the magnitude of the slow and the fast components (M_i and M_o) are shown in Fig. 3. When the perfusion rate was increased, the fast component ($M_o = (1-H)M$) increased significantly wheras the slow component ($M_i = HM$) remained virtually unchanged.

In the second experiment, four glands were perfused with a hypotonic perfusate (with the concentration of NaCl reduced by 80 mmol l^{-1}) in order to expand the intracellular volume. The water compartment responsible for the slow component almost doubled in size ($N_i = 0{\cdot}56 \pm 0{\cdot}06$ g, $n = 4$) compared with control values (Table 1). There were also significant changes in the rate constants k_x and k_y: the

value of k_x decreased to $3{\cdot}1 \pm 0{\cdot}2$ s^{-1}, and k_y increased to $1{\cdot}2 \pm 0{\cdot}1$ s^{-1}. These changes are consistent with the increase in the ratio of the intracellular and extracellular volumes (H increased to $34{\cdot}3 \pm 1{\cdot}8$ %).

Finally, one gland was perfused with a hypertonic perfusate in order to reduce the size of the intracellular space. As shown in Fig. 4, addition of sucrose (500 mmol l^{-1})

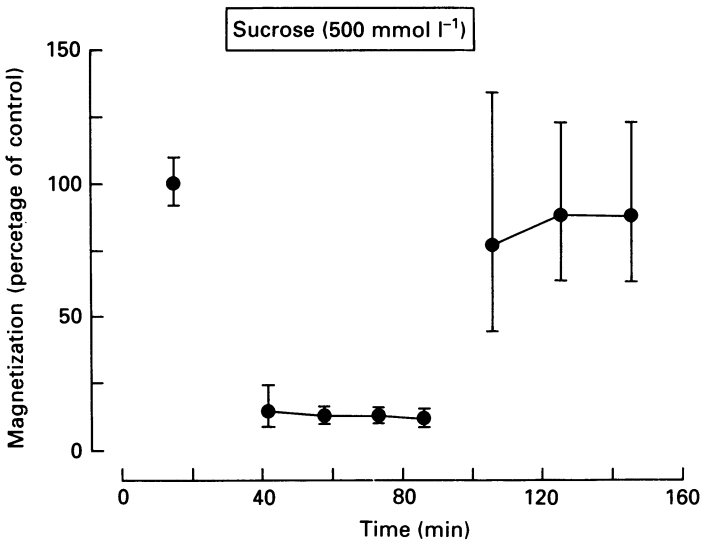

Fig. 4. Changes in the initial magnetization due to the slow component of relaxation (M_i) in a single gland when the perfusate was made hypertonic by addition of 500 mmol l^{-1} sucrose. Values are the zero-time intercepts of the slow component of relaxation obtained by least-squares regression analysis of full sets of relaxation data and are normalized to the initial control value. Error bars show the 95% confidence limits of the regression coefficients.

caused a large decrease in the size of the slow component (M_i). When the osmolarity of the perfusate was restored to its control value after 60 min, M_i returned to its original value.

Thus, the results of all three experiments support the assignment of the slow and fast relaxation components to the intracellular and extracellular water populations respectively.

Effects of acetylcholine stimulation

Water proton relaxation rate and the intracellular water content of the gland were also measured during stimulation with acetylcholine at a concentration (10^{-6} mol l^{-1}) that is optimal for sustained fluid secretion. After a period of control perfusion lasting 60 min, acetylcholine was administered for 30 min, and then the perfusion was continued without acetylcholine for a further 35 min. Values of ϕ_A, ϕ_B, H and M were measured before, during and after stimulation, using relaxation data collected during the last 10 min of each period.

As reported previously (Case *et al.* 1980), the secretory rate reaches an almost constant (plateau) value after about 20 min of continuous stimulation with

acetylcholine. The relaxation data that were obtained during stimulation therefore reflect the state of the gland during the first part of the plateau phase of secretion. None of the parameters showed any significant change compared with the data from the unstimulated gland (Table 1).

DISCUSSION

In rabbit mandibular salivary glands perfused with 10 mmol l^{-1} Gd(DTPA), water proton relaxation separated into two exponential components. The faster of the two components contributed 81% of the total NMR signal and had a rate constant similar to that of the perfusate. This component most probably originated from the large extracellular water content of the oedematous gland. Since water exchange across the capillary wall is extremely rapid (Gamble, Smaje & Spencer, 1988), the intravascular perfusate and interstitial fluid would be expected to behave as a single compartment.

The slower component of relaxation (19% of the total water signal), although not as rapid as the extracellular component, had a relaxation rate that was an order of magnitude greater than that of glands perfused without the relaxation reagent. Several observations point to the origin of this component in the intracellular water of the acinar cells: (1) the magnitude of the slow component corresponded to a sizeable fraction (71%) of the wet weight of the gland after removal of the oedematous connective tissue by dissection; (2) it was unaffected by expansion of the extracellular space by increased perfusion pressure; (3) it increased and decreased in the expected fashion when the perfusate osmolarity was altered.

The increase in the apparent relaxation rate of the intracellular water protons in the presence of extracellular relaxation reagent can be attributed to diffusive water exchange between the two compartments, as observed previously in other tissues (Fabry & Eisenstadt, 1975; Steward & Garson, 1985; Verkman & Wong, 1987). An alternative possibility, the penetration of Gd(DTPA) or free Gd^{3+} ions into the cells, can be discounted on the grounds that the relaxation time course did not alter with the duration of the perfusion. The relaxation reagent used in this study is a very stable complex of Gd^{3+} (Perrin, 1979) and it is unlikely that it crosses the cell membranes or alters their permeability properties. Indeed, glands perfused with 10 mmol l^{-1} Gd(DTPA) and stimulated with 10^{-6} mol l^{-1} acetylcholine secreted fluid at rates comparable with those obtained in the absence of the relaxation reagent. In the presence of a very small excess of free Gd^{3+}, however, the flow rate during the plateau phase of the secretory response was greatly depressed (J. M. Rawlings & M. C. Steward, unpublished data), probably as a result of an inhibitory effect of Gd^{3+} on calcium influx rather than a direct effect on the water permeability of the membranes.

Estimation of diffusive water permeability P_d

On the assumption that the time course of the slow component of relaxation was mainly determined by the rate at which intracellular water molecules underwent diffusive exchange with the extracellular population, we have calculated the efflux rate constant k_x in the unstimulated gland to be $4{\cdot}1 \pm 0{\cdot}1$ s^{-1} ($n = 16$). To convert this

parameter to a diffusive permeability coefficient, expressed per unit area of membrane, requires information about the route of water efflux from the cells, i.e. an estimate of the relative contributions of the basolateral and luminal membranes. Although a full stereological analysis of this tissue is not available, it is evident from morphological studies (Young & van Lennep, 1978; Toyoshima & Tandler, 1986) that the area of the basolateral surface of the acinar cells greatly exceeds that of the luminal surface. The basolateral surface is therefore likely to contribute the major pathway for water efflux. If, however, the luminal membrane has a proportionally greater water permeability per unit area, its contribution may also be significant. Efflux via this route will only be detected if Gd(DTPA) is present in the luminal fluid – unlikely in view of the size and charge on the complex – or if there is rapid water exchange across the junctional complex so that the luminal and interstitial fluids behave as a single compartment. The latter possibility cannot be ruled out in view of the presumed leakiness of the paracellular pathway, at least to monovalent cations (Cook & Young, 1989).

Using measurements from the electron micrographs of Toyoshima & Tandler (1986), we have estimated the surface area and volume of a single acinar cell by assuming that the geometry of the cell can be approximated by a 120 deg sector of a cylinder of radius 20 μm and length 20 μm. The surface area (A) (not allowing for surface amplification due to luminal microvilli and basolateral folds) will thus be approximately 2500 μm^2, and the volume (V) approximately 17000 μm^3. Using these values, the apparent diffusive water permeability of the whole surface membrane,

$$P_d = k_x V/A, \tag{5}$$

is estimated to be approximately 3×10^{-3} cm s^{-1}. If the NMR method only detects efflux through the basolateral membrane, this figure will be a good estimate of the basolateral membrane permeability because the value of A is dominated by the basolateral surface area. If there is a significant, detectable water efflux through the luminal membrane, the true figure for the basolateral membrane will be smaller.

The value of 3×10^{-3} cm s^{-1} obtained for P_d is similar to values obtained for *Necturus* gallbladder ($1{\cdot}6 \times 10^{-3}$ cm s^{-1}; Steward & Garson, 1985), rabbit proximal tubule (2×10^{-3} cm s^{-1}; Verkman & Wong, 1987) and human erythrocytes (3·3 to $4{\cdot}7 \times 10^{-3}$ cm s^{-1}; Herbst & Goldstein, 1989). It lies towards the top end of the range of values characteristic of animal cell membranes (House, 1974) and artificial phospholipid membranes and liposomes (Fettiplace & Haydon, 1980).

Effects of acetylcholine stimulation

Secretion by the rabbit mandibular gland is mainly controlled *in vivo* by the muscarinic agonist, acetylcholine. Stimulation of the gland with acetylcholine brings about marked changes in the permeability of the acinar cell membranes to certain electrolytes (reviewed by Cook & Young, 1989) and significant changes in intracellular pH (Steward, Seo & Case, 1989), but no information has been available hitherto about the possibility of changes in the permeability of the membranes to water.

The results of the present study indicate that continuous stimulation with acetylcholine, at a concentration that is known to elicit fluid secretion, has little effect on the rate constant for diffusive water efflux k_x, suggesting that there is no

significant change in the water permeability of the membranes. We cannot exclude the possibility that there are changes in the permeability of the luminal membrane if, as is possible, water efflux by this route is not detected by the NMR method. In addition, if one of the two membranes had a very much greater permeability than the other, changes in the permeability of the less permeable membrane might not be readily detected.

The apparent lack of effect of acetylcholine is not altogether surprising. Unlike the distal segments of the renal tubule, the control of water transport across the salivary epithelium appears to be achieved by regulation of the driving force (i.e. the rate of electrolyte transport), thus there is no need for the water permeability of the membranes to change.

Osmotic driving forces required for secretion

To estimate the osmotic water permeability P_f from the diffusive water permeability P_d requires some assumptions to be made about the mechanism of osmotic water flow (Hill, 1979, 1982; Finkelstein, 1987). If osmotic water flow through the membrane is by simple diffusion, the two parameters should be equal. If water flow is viscous, i.e. driven by a pressure gradient within an aqueous pore, P_f may exceed P_d by a factor which depends upon the geometry of the pore. However, for reasons which have been discussed previously (Steward & Garson, 1985), the ratio P_f/P_d is unlikely to exceed a value of approximately 5. Larger values found in the literature can generally be attributed to unstirred-layer artifacts (Barry & Diamond, 1984), or to single-file water flow through long, narrow pores (reviewed by Finkelstein, 1987). Since small non-electrolytes can be entrained in the water flow across the acinar epithelium (Case *et al.* 1985), it is unlikely that the latter situation arises in this tissue.

For the purpose of predicting the osmotic gradients that would be required to drive water across the transcellular pathway during secretion, it is more convenient to calculate the diffusive water permeability per gland,

$$P'_d = k_x N_i, \tag{6}$$

rather than per unit area of membrane. This calculation makes use of the measured intracellular water content, N_i, which should be a reasonable estimate of the intracellular volume per gland, and obviates the need for estimating the total epithelial surface area.

If we suppose that the efflux of water measured by proton relaxation occurs entirely across the basolateral membrane of the acinar cells, we calculate the diffusive water permeability per gland, P'_d, to be 1·2 $cm^3\ s^{-1}$. This suggests that the osmotic permeability of this membrane per gland, P'_f, lies in the range from 1·2 to 6·2 $cm^3\ s^{-1}$ (assuming P'_f/P'_d lies between 1 and 5). Since water flow (J_v, $cm^3\ s^{-1}$) is related to the osmotic gradient across the membrane (ΔC, osmol l^{-1}) by the expression:

$$J_v = (P'_f \bar{V}_w \Delta C) \times 10^{-3}, \tag{7}$$

where $\bar{V}_w$ is the partial molar volume of water (18 $cm^3\ mol^{-1}$), it is possible to estimate the osmotic gradient required to drive a given flow of water.

During continuous stimulation with 10^{-6} mol l^{-1} acetylcholine, the secretory rate of the perfused gland declines from an initial value in excess of 250 $\mu l\ min^{-1}$

($4{\cdot}2 \times 10^{-3}$ cm^3 s^{-1}) to a plateau rate of approximately 40 μl min^{-1} ($0{\cdot}7 \times 10^{-3}$ cm^3 s^{-1}) over a period of about 20 min (Case *et al.* 1980). Micropuncture studies of the rat mandibular gland (Martinez, Holzgreve & Frick, 1966; Young & Schögel, 1966) have shown that the fluid that forms the primary secretion of the gland is approximately isosmotic with plasma.

Using our maximum estimate of P_f' (6·2 cm^3 s^{-1}), the osmotic gradients needed to drive the observed initial and plateau flows across the basolateral membrane would be 37·6 and 5·9 mosmol l^{-1}, respectively. Since there would also need to be an osmotic gradient across the luminal membrane, it is difficult to see how the primary secretion could approach isotonicity, particularly at the higher flow rates.

If, as suggested earlier, the measured rate constant for water efflux includes a component of efflux across the luminal membrane, the water permeability of the basolateral membrane will have been overestimated in these calculations. This means that the osmotic gradient needed to drive water across the basolateral membrane would need to be even greater than the values calculated above.

We conclude, therefore, that if the osmolarity of the primary secretion lies within a few mosmol l^{-1} of the perfusate osmolarity, as is widely believed (although perhaps not adequately proven), the water permeability of the basolateral membrane is insufficient to account for more than a small fraction of the observed water flow. This would suggest that the bulk of the secreted water crosses the epithelium by a paracellular pathway. However, it remains possible that the primary secretion is indeed hypertonic, and only approaches isotonicity as it flows through the terminal branches of the duct system – in effect, a luminal, standing-gradient osmotic flow system (Diamond & Bossert, 1967).

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