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A model for fluid secretion in the exocrine pancreas

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Fluid secretion by the isolated rabbit pancreas is strongly dependent on the presence of Na^+ in the bathing medium. Substitution of Na^+ by another cation such as Li^+ or K^+ causes an inhibition of fluid secretion rate and a change in the composition of the secreted fluid which is dependent on the nature of the substituent cation. Stimulation of the pancreas by CCK-8 or carbachol increases paracellular ion permeability and, in some cases, also fluid secretion rate. We present a simple, quantitative model for ion and water secretion which accounts for the effects observed upon Na^+ substitution and stimulation. The main features are active, Na^+-dependent transcellular HCO_3^- transport and passive, paracellular cation and anion permeation. The activity of the HCO_3^- pump is dependent on the energy status of the cell and on the Na^+ concentration in the bathing medium, and is competitively inhibited by K^+. The paracellular ion permeabilities can be modulated by stimulatory agonists. We examine the extent to which, according to the model, fluid secretion is controlled by the various system parameters such as ion permeabilities and ion pump activity, and by external parameters such as the ion concentrations in the bathing medium. In addition, calculation of the effects of changes in these parameters are carried out in order to gain more insight in the mechanisms of secretion.

Introduction

Fluid secretion is generally thought to be the result of an osmotic gradient generated by the transport of ions across a membrane or epithelium [1]. In the exocrine pancreas, the primary uphill transported ion is the HCO_3^- ion which is concentrated in the secreted fluid by a factor of 1 to 5 as compared to the bathing or perfusion fluid [2,3]. All the other major ions in the secreted fluid are present in concentrations equal to or lower than those in the medium. A variety of studies on pancreatic fluid secretion have shown that, besides HCO_3^-, Na^+ is essential for secretion [3–6]. The other ions K^+, Cl^-, $H_2PO_4^-$ and Ca^{2+} and Mg^{2+} are probably not directly involved in HCO_3^--dependent fluid secretion.

It is likely that Na^+ and the other mono- and divalent cations are secreted through a paracellular route [6]. Since Na^+ is the most abundant cation in the medium and in the secreted fluid, one might argue that the role of Na^+ is maybe essential, but only passive. However, removal of Na^+ has approximately the same effect on ion and water secretion as the removal of HCO_3^- and therefore Na^+ and HCO_3^- seem to interact with the same active transport mechanism [7]. One way to determine whether, or to what extent, the role of Na^+ is to activate the HCO_3^- pump or to allow for paracellular ion movement, would be to replace Na^+ with other ions with different shunt permeabilities. In the previous paper we have reported the effects of substituting Li^+, K^+ or choline for Na^+ on the fluid secretion rate and the ion concentrations in the secreted fluid. In a qualitative discussion we tentatively concluded that the effects of the substitutions could not easily be explained by the different permeabilities of the shunt for the different ions alone, or by a simple Na^+ dependence of the HCO_3^- pump alone [8].

To assess the relative importance of the various ways in which the Na^+ concentration would affect secretion, a quantitative formulation of the model for secretion is needed. Such a formulation can also allow us to determine e.g., whether the transepithelial electrical potential difference, the Cl^- permeability, or the simple pumping of HCO_3^- is pivotal to fluid secretion. In the preceding paper we have reported the effects of the

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messengers acetylcholine (ACh), or its analogue carbachol, and cholecystokinin (CCK), or its octapeptide (CCK-8), on fluid and ion secretion in the pancreas [8]. Analysis of these effects in terms of a model would also enable us to further define their mechanism of action.

In order to establish how a metabolic process such as secretion can be modulated by the partial activation or inactivation of enzymes or permeabilities, metabolic control theory has generated concise definitions of so-called control coefficients [9–12]. From a quantitative formulation of pancreatic fluid secretion one would be able to evaluate these control coefficients and begin to understand to what extent each of the fundamental processes controls and thus regulates overall secretion.

For modelling of transepithelial transport various methods exist. We expected the phenomenological non-equilibrium thermodynamic method [13] to lack detail to allow us to address the rather mechanistic questions asked above. A second method, i.e. the complete integration of all the exact rate equations of all the processes involved [14], would lack sufficient detailed kinetic information. Thus we employed an approach in-between these two [12], in which simple rate equations are used which nevertheless bear the essential properties of non-equilibrium thermodynamics and kinetics in them, and which can be solved numerically. The equations can also be solved in terms of network thermodynamics as has been done successfully for kidney tubules [15–17].

In this paper we describe the model and calculate the parameter values that bring the model in line with the experimental findings. Subsequently, we determine to what extent the rate of fluid secretion and the composition of the secreted fluid are controlled by the active HCO_3^- secretion and the various paracellular ion permeabilities. Finally, we use the model to get more insight in the role of the various mechanisms involved in fluid secretion.

Methods

In this section we will go through the basic formulas which make up the model, and which we have found to be the most appropriate for this particular epithelium. The equations which relate the flow of anions or cations to their permeability coefficients and concentrations and to their active pump components are as follows:

$$J_{HCO_3^-} = J_V[HCO_3^-]_s = \text{act}\cdot f_{Na^+}\cdot(K[HCO_3^-]_b - \phi[HCO_3^-]_s) + (P_{HCO_3^-} + \alpha P_{HCO_3^-m})([HCO_3^-]_b - \phi[HCO_3^-]_s) \quad (1)$$

$$J_{Na^+} = J_V[Na^+]_s = (P_{Na^+} + \alpha P_{Na^+m})(\phi[Na^+]_b - [Na^+]_s) \quad (2)$$

$$J_{C^+} = J_V[C^+]_s = (P_{C^+} + \alpha P_{C^+m})(\phi[C^+]_b - [C^+]_s) \quad (3)$$

$$J_{Cl^-} = J_V[Cl^-]_s = (P_{Cl^-} + \alpha P_{Cl^-m})([Cl^-]_b - \phi[Cl^-]_s) \quad (4)$$

$$f_{Na^+}([Na^+]_b, [C^+]_b) = [Na^+]_b K_{Na1}\frac{1}{1+\frac{[C^+]_b}{K_{pi}}} \quad (5)$$

Explanation of symbols:

J_V = transepithelial volume (= water) flow

J_I = transepithelial flow of ion I

$[I]_{b,s}$ = concentration of ion I in bathing medium or secreted fluid, respectively

C^+ = cation replacing Na^+

K = apparent equilibrium constant of HCO_3^- transport mechanism, or the driving force of the HCO_3^- pump

act = pump activity; a complex function of various cellular activities

P_I = shunt permeability coefficient of ion I

P_{Im} = modulated shunt permeability coefficient of ion I for the stimulant induced ion channel

α = factor proportional to the degree to which the stimulant activated paracellular shunt is open

ϕ = measure for the transepithelial electric potential difference, $\exp(F\Delta\psi/RT)$

$\Delta\psi$ = transepithelial electrical potential difference (bath relative to secreted fluid)

K_{Na1} = Michaelis constant of the HCO_3^- pump with respect to Na^+

K_{pi} = inhibition constant of the HCO_3^- pump with respect to the cation substituting for Na^+

The above equations describe the model depicted in Fig. 1B, which is a reduction of the model given in Fig. 1A. This reduction recognizes that, essentially, the overall system consists of two elements in parallel, one, the cell or cell membranes, actively pumping HCO_3^- across the epithelium, the other, the paracellular path, allowing passive transepithelial permeation of water and all the ions. The first equation (Eqn. 1) describes the active pumping of HCO_3^- as well as the passive HCO_3^- permeation. Eqns. 2–4 describe the passive movements of Na^+, cations and Cl^-, respectively, across the epithelium.

The HCO_3^- pump, functioning at a rate $J_p^{HCO_3^-}$ (Eqn. 1), is driven by an effective thermodynamic driving force:

$$\Delta G_{in}/RT = \ln K \quad (6)$$

which is likely to be a complex function of the activity of various cellular processes, such as the Na^+/K^+-ATPase and Na^+/H^+ antiport or Na^+/HCO_3^- symport activity across the basolateral membrane, and of the intracellular hydrolytic free energy of ATP. The HCO_3^- concentration in the bath, or, effectively, at the active site of the HCO_3^- translocator is assumed to be constant and buffered by pH and p_{CO_2}. The factor f_{Na^+} (Eqns. 1 and 5) describes the modulation of the activity

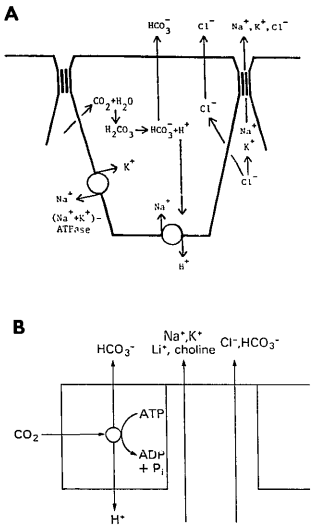

Fig. 1. (A) Conceptual model for fluid secretion in the ductular cell of the exocrine rabbit pancreas. In essence, the Na^+/K^+-ATPase drives a coupled Na^+/H^+ antiport (or Na^+/HCO_3^- symport) mechanism through the establishment of the Na^+ gradient, which, through intracellular alkalinisation and production of HCO_3^- maintains the transcellular transport of HCO_3^-, Na^+ and K^+, and other cations, are secreted via the paracellular pathway. The transport route for Cl^- is paracellular, transcellular or both. Omitted are the leakage pathways for the various ions, such as for K^+ in the basolateral membrane. (B) Model for fluid secretion in the pancreas as a result of simplification of Fig. 1A through a nonequilibrium thermodynamics treatment. The model features an electrogenic transepithelial HCO_3^- pump furnished by the ductular cells, parallel to paracellular, passive cation and anion movement.

of the pump by the bath concentrations of Na^+ and the substituting cations. In our main model, the choices of the constants are such that the right hand side of Eqn. 5 contains $[K^+]_b$ as the only relevant cation concentration term ($[C^+]_b$). Thus the pump activity is taken to be proportional to the Na^+ concentration in the bath and to be competitively inhibited by K^+ in the bath.

As witnessed by the last right hand term in Eqn. 1, HCO_3^- may also move through the paracellular pathway, with a thermodynamic driving force betrayed by the near equilibrium relationship:

$$J_{HCO_3^-}^{shunt} = L_{HCO_3^-}\left(RT \ln \frac{[HCO_3^-]_b}{[HCO_3^-]_s} - Fd\psi\right) \quad (7)$$

where $L_{HCO_3^-}$ would be proportional to the average HCO_3^- concentration in the shunt [12,13]. We used the related thermokinetic relationship given in the last right hand term of Eqn. 1, which reduces to Eqn. 7 in the near equilibrium case. $P_{HCO_3^-}$ is the permeability coefficient of the shunt pathway for HCO_3^- in the absence of a stimulus. $P_{HCO_3^-,m}$ represents the specific increase in HCO_3^- permeability when the shunt is activated by a stimulus. Similar rate equations are assumed to hold for the paracellular movement of Na^+, Cl^- and the substituting cations (Eqns. 2–4).

It may be noted that the electric driving force has been brought into the rate equations solely as a positive exponent. Although this is an arbitrary choice [18], it has little implication due to the fact that the paracellular transport of cations and Cl^- is close to equilibrium.

The right hand sides of Eqns. 1–4 contain six variables, i.e. the four ion concentrations in the secreted fluid, the water or volume flow J_V and the electrical potential factor ϕ. An additional expression for the volume flow is [19]:

$$J_V = L_P\left(\Delta P - \sum_i \sigma_i \Delta\pi_i\right) + V_{HCO_3^-} J_P^{HCO_3^-} \quad (8)$$

Here L_P is the hydraulic permeability coefficient for water flow, ΔP is the transepithelial difference in hydrostatic pressure, which is equal to zero in the cases under consideration, σ_i is the reflection coefficient for any substance i (in our case NaCl and $NaHCO_3$) [19,20], and $\Delta\pi_i$ is RT times the transepithelial concentration difference for substance i. The term containing the partial molal volume of HCO_3^- ($V_{HCO_3^-}$) and the HCO_3^- flux through the pump ($J_P^{HCO_3^-}$) can be neglected. We will now assume that the water permeability and hence L_P is high compared to the activity of the HCO_3^- pump and that the reflection coefficients of the various solutes are approximately equal, if not all close to 1. The consequence is that the osmotic pressure, $\Delta\pi$, across the epithelium is negligible, as is observed experimentally [3,21]. We will also assume electroneutrality of both the bath and the secreted fluid which, together with the neglection of $\Delta\pi$, leads to:

$$[Cl^-]_s + [HCO_3^-]_s = \pi_{bath}/2 = [Na^+]_s + [C^+]_s \quad (9)$$

Using the latter two equations, Eqns. 1–4 are reduced to four equations with four unknowns, i.e. J_V, ϕ, $[Na^+]_s$ and $[HCO_3^-]_s$, and solved numerically.

In the equations used for the simulations, all the ion concentrations in the bathing medium and secreted fluid are normalized, so that the sum of cation concentrations = 1, and the sum of anion concentrations = 1. We also assumed that the only cations are Na^+ and K^+ and replacing cations, and that the only anions are Cl^- and HCO_3^-. In this procedure, we did not dis-

criminate between Na^+ and K^+ in the normal, Li^+ and choline media, since K^+ is present in only minor amounts and the permeability for K^+ is virtually equal to that for Na^+ [22]. Thus, $[Na^+]_b$ in the model equations is equal to the measured $[Na^+ + K^+]_b$ value, except for the media in which Na^+ is replaced by K^+, where $[Na^+]_b$ in the equations is the measured $[Na^+]_b$ value. Parameter values for the model were obtained by attempting to find the best possible non-linear least-squares fit to the data presented in the previous paper (in Table I). The fitted data were the volume flow, the Na^+ concentration in the secreted fluid and the Cl^- concentration in the secreted fluid. In view of the possible exchange between Cl^- and HCO_3^- in the efferent ducts we only attempted to reproduce the trends in the Cl^- concentration and J_{Cl^-}. Furthermore, we assumed that CCK-8 and carbachol increase the ion permeability by opening an extra paracellular shunt pathway to an extent dependent on the stimulant concentration reflected in the factor α. This extra shunt pathway has different permeability ratios for the various ions than the normal pathway. The cation permeabilities of this pathway were taken equal, and higher than the anion permeabilities.

The extent to which certain enzymes are rate-limiting for a metabolic flux can be quantified in terms of their flux-control coefficients [23]. The flux control coefficient indicates the relative effect on the flux of a small relative change in the enzyme activity. It is defined mathematically as:

$$C_{e_i}^{J} \equiv (dJ/J)/(de_i/e_i) = 100 \cdot (\Delta J/J)_{1\%\ \text{increase in}\ e_i}$$

$$= (\%\ \text{increase in}\ J)_{1\%\ \text{increase in}\ e_i} \qquad (10)$$

where the differential refers to transitions between steady states, and e_i refers to the activity of the enzyme under study. More in general, e_i can be replaced by any parameter of the system, such as permeabilities, equilibrium constants or fixed concentrations. This method was applied to calculate the control coefficients of, e.g., ion permeabilities, act and K with respect to variables such as J_V or $[Na^+]_s$. In Eqn. 10 it can be seen that a control coefficient of 1 represents a proportional dependence of J on the relevant e_i.

Results

Summary of experimental findings

The data on the effects of replacement of Na^+ by Li^+, K^+ or choline on fluid secretion rate and ion concentrations in the secreted fluid have been described in the accompanying paper (Ref. 8, Table I). When we normalize the data from control, Li^+ (74 mM), choline (66 mM) and K^+ (99 mM) substitution experiments and

TABLE I

Experimental and model results for pancreatic fluid secretion in various media

Pancreatic fluid secretion rate, ion composition and ϕ were determined experimentally or calculated according to our model under control conditions, or when Na^+ in the bathing medium was replaced by Li^+, choline or K^+, and when the pancreas was stimulated by CCK-8 (all conditions). Results of the model are given in italics. J_V represents fluid secretion rate, J_I is the flow rate of ion I, and $[I]_{b,s}$ the concentration of ion I in bathing medium or secreted fluid. C^+ is the Na^+ replacing cation. ϕ equals $e^{F\Delta\psi/RT}$. $\Delta\psi$ represents the electrical potential across the epithelium. The experimentally determined value of ϕ is $[K^+]_s/[K^+]_b$ and is given in parentheses. J_V is expressed as percentage of the control value before Na^+ replacement or stimulation. $[I]_{b,s}$ values are normalized values, so that the sum of cation and anion concentrations in bath or secreted fluid equals 1. $[Na^+]_{b,s}$ represents $[Na^+ + K^+]_{b,s}$, except when Na^+ is replaced by K^+.

Medium	$[Na^+]_b$	$[C^+]_b$	J_V	$[Na^+]_s$	J_{Na^+}	$[C^+]_s$	J_{C^+}	$[Cl^-]_s$	J_{Cl^-}	$[HCO_3^-]_s$	$J_{HCO_3^-}$	ϕ
Control	1	0	100	1	100	0	0	0.47	47	0.53	53	(1.28)
			100	*1*	*100*	*0*	*0*	*0.52*	*52*	*0.48*	*48*	*1.13*
+CCK-8	1	0	104	1	104	0	0	0.53	55	0.47	49	(0.91)
			105	*1*	*104*	*0*	*0*	*0.54*	*56*	*0.46*	*48*	*1.10*
Li^+	0.50	0.50	54	0.54	29	0.46	25	0.52	28	0.48	26	(2.02)
			54	*0.58*	*31*	*0.42*	*23*	*0.57*	*30*	*0.44*	*23*	*1.26*
+CCK-8	0.50	0.50	61	0.50	31	0.50	30	0.59	36	0.41	25	(1.46)
			61	*0.52*	*32*	*0.48*	*23*	*0.62*	*38*	*0.38*	*23*	*1.11*
Choline	0.56	0.44	45	0.92	41	0.08	3.6	0.51	23	0.49	22	(1.51)
			45	*0.93*	*42*	*0.07*	*3.2*	*0.44*	*20*	*0.56*	*25*	*1.77*
+CCK-8	0.59	0.41	52	0.79	41	0.21	11	0.56	29	0.44	23	(1.21)
			52	*0.78*	*40*	*0.22*	*11*	*0.51*	*26*	*0.49*	*25*	*1.47*
K^+	0.35	0.65	40	0.29	15	0.61	25	0.75	30	0.25	9.9	(0.98)
			41	*0.35*	*14*	*0.65*	*26*	*0.69*	*28*	*0.31*	*12*	*1.05*
+CCK-8	0.36	0.64	43	0.35	15	0.65	28	0.79	34	0.21	9.2	(0.97)
			43	*0.35*	*15*	*0.65*	*27*	*0.71*	*30*	*0.29*	*12*	*1.04*

add the numbers for ion flows we obtain the data shown as arabic numbers in roman type in Table I. The basic observations are:

(i) The concentration ratios for the various cations between secreted fluid and bathing medium suggest that the permeabilities for Na^+ and K^+ are about equal and those for Li^+ and choline are lower than for Na^+: $P_{Na^+} \approx P_{K^+} \geq P_{Li^+} \geq P_{choline}$. Hence there is probably no inhibitory effect of substitution of Na^+ by K^+ due to a passive permeability effect. Li^+ and choline might inhibit fluid secretion partly because of their lower permeability coefficients.

(ii) Stimulation by CCK-8 of fluid secretion rate (J_V) in the Li^+ and choline medium is larger than in normal or K^+ medium.

(iii) In the case of K^+ or Li^+ substitution, the fluid secretion rate J_V is nearly linearly inhibited, i.e., J_V is virtually proportional to $[Na^+]_s$. However, the HCO_3^- flow ($J_{HCO_3^-}$) is inhibited linearly in the case of Li^+ substitution, but more strongly in the case of substitution of choline for Na^+ and even more in the case of substitution of K^+ for Na^+.

(iv) If we take the K^+ concentration ratio between secreted fluid and bathing medium $[K^+]_s/[K^+]_b$ as an indicator of the transepithelial electrical potential difference, we can see that this potential is increased upon replacement of Na^+ by a less permeant cation (choline, Li^+), and decreased upon stimulation by CCK-8 in all cases.

A model that simulates the experimental results

At first, we attempted to simulate the data with a variant of the model described in Methods, in which the HCO_3^- pump activity was taken to be independent of the Na^+ concentration in the bath ($f_{Na^+} = 1$) and the shunt permeabilities of the substituting cations were different from that of Na^+. Then we tried another simulation by using a model in which the pump activity was dependent on the Na^+ concentration in the bath, but in which the permeabilities of the various cations were identical. Since both variants of the model were not able to simulate the experimental findings summarized in Table I, we turned to a combination model which has become the main model of this paper.

The parameter values for this model, as it is described in Methods, are given in Table II. The values for fluid and ion secretion rates predicted by the model are given in Table I (*italics*), and are within approx. 10% of the experimental values. The results on $[Cl^-]_s$ and J_{Cl^-}, and on $[HCO_3^-]_s$ and $J_{HCO_3^-}$ are generally somewhat more deviating from the experimental results than those on J_V, $[Na^+]_s$ or $[C^+]_s$ and J_{Na^+} or J_{C^+}. $[Cl^-]_s$ is slightly increased in choline medium, whereas the model predicts a decrease. However, both in experimental and model situation, $[Cl^-]_s$ is lower in choline and Li^+ medium than in K^+ medium, which seems to be correlated to $\Delta\psi$, which is lowest in K^+ medium. Part of the discrepancy in the results on $[Cl^-]_s$ could be due to an electroneutral $Cl^- - HCO_3^-$ exchange in the ductal system which would tend to increase $[Cl^-]_s$ at lower flow rates. In the case of Li^+ substitution, however, this argument does not suffice since $[Cl^-]_s$ (0.52) is lower than the value predicted by the model (0.57).

TABLE II

Parameter values for quantitative model of pancreatic fluid secretion

Values were determined by an iterative method to give the best possible fit of the model to the experimental results. K is the apparent equilibrium constant, act is the maximum activity of the pump, P_i is the ion permeability coefficient of ion I under control conditions, and P_{Im} is the stimulus induced permeability coefficient when secretion is modulated by the stimulus CCK-8. K_{pi} is the inhibition constant with respect to the substituting cation in case of Na^+ replacement. $K_{Na,I}$ is the Michaelis constant of the pump with respect to Na^+, and α is a constant relating P_{Im} to J_I, and is proportional to the 'openness' of the activated shunt pathway. In line with the experimental situation [8], $[Cl^-]_b$ was set to 0.895.

Pump	$K = 90$ act = 55.05		
Shunt permeabilities	$P_{Na^+} = 755$ $P_{K^+} = 755$ $P_{Li^+} = 105$ $P_{chol} = 4.46$ $P_{Cl^-} = 167$ $P_{HCO_3^-} = 2.03$	CCK-8 induced permeabilities ($\alpha = 0.02707$)	$P_{Na^+,m} = 10000$ $P_{K^+,m} = 10000$ $P_{Li^+,m} = 10000$ $P_{chol,m} = 800$ $P_{Cl^-,m} = 800$ $P_{HCO_3^-,m} = 33.3$
Constants	$K_{pi}(chol, Li^+) = 100000$ $K_{pi}(K^+) = 2$ $K_{Na,I} = 0.01$		

Control coefficients

The control coefficients (C) of act, the ion permeabilities, K, the ion concentrations in the bath, and of α, all with respect to J_V, ϕ, $[Cl^-]_s$ and $[Na^+]_s$ have been calculated for normal medium (Table III) and for choline medium (Table IV). From the results of Table III, it can be concluded that the pump activity and, to a minor extent, the Cl^- permeability are important in the control of J_V ($C^{J_V}(act) = 0.70$ and $C^{J_V}(P_{Cl^-}) = 0.26$). The low, negative value of the control coefficient of $P_{HCO_3^-}$ with respect to J_V (-0.02) indicates that an increase of $P_{HCO_3^-}$ under these conditions would slightly decrease J_V. The second column in Table III shows that, according to the model, the anion concentration profile in the secreted fluid is mostly determined by act ($C^{[Cl^-]_s}(act) = -0.30$) and P_{Cl^-} ($C^{[Cl^-]_s}(P_{Cl^-}) = 0.24$) and less so by P_{Na^+} or $P_{HCO_3^-}$. The transepithelial potential, or ϕ, is mainly controlled by the Na^+ permeability ($C^{\phi}(P_{Na^+}) = -0.11$) and the pump activity ($C^{\phi}(act) = 0.08$). It can be seen that the sum of the control coefficients of the independent activity parameters (act, ion permeabilities and α) with respect to the fluxes such as J_V is 1,

TABLE III

Control of system parameters on fluid secretion in normal bathing medium

Values of control coefficients were determined according to Eqn. 10, and range from 0 (no control) to ±1 (complete control). System parameters are act, P_{Na^+}, P_{Cl^-}, $P_{HCO_3^-}$ and α, and the sum of the control coefficients of these parameters is 1 or 0 as given in the table. The control coefficients of K and $[Cl^-]_b$ (the latter being related to the coefficient of $[HCO_3^-]_b$ since the sum of these anion concentrations is constant), which are parameters other than the intrinsic system parameters, are given in the lower part of the Table. J_V is the fluid secretion rate, $[Cl^-]_s$ secretory Cl^- concentration, and ϕ is equal to $e^{F\Delta\psi/RT}$, where $\Delta\psi$ is the electrical potential across the epithelium. The control coefficients which are noteworthy are underlined. The subscript m refers to the CCK-8-stimulated state.

Control coefficient (C) of	C with respect to			C with respect to		
	J_V	$[Cl^-]_s$	ϕ	J_{Vm}	$[Cl^-]_{sm}$	ϕ_m
act	0.70	−0.30	0.08	0.70	−0.27	0.06
P_{Na^+}	0.06	0.05	−0.11	0.04	0.03	−0.06
P_{Cl^-}	0.26	0.24	0.03	0.24	0.20	0.02
$P_{HCO_3^-}$	−0.02	0.01	> −0.01; < 0	−0.01	0.01	> −0.01; < 0
α	0	0	0	0.04	0.04	−0.02
Sum	1	0	0	1	0	0
K	0.70	−0.29	0.08	0.70	−0.28	0.06
$[Cl^-]_b$	0.75	0.68	0.09	0.82	0.67	0.08

and the sum of these coefficients with respect to $[Cl^-]_s$ and ϕ is 0, as they should be [9,10,12,23].

The control coefficients of K and $[Cl^-]_b$ are different in nature from those of act, the ion permeabilities and α, since the former parameters affect the fluxes in the system via the latter ones. The control coefficient of $[Cl^-]_b$ represents in effect the control by the interdependent ion concentrations of Cl^- and HCO_3^-, since $[Cl^-]_b$ cannot be changed without changing also $[HCO_3^-]_b$ as we work within the limitation of constant medium osmolarity. The magnitudes of the control coefficients of K and $[Cl^-]_b$ with respect to J_V, $[Cl^-]_s$ and ϕ are all relatively large. That is [12], fluid secretion rate, $[Cl^-]_s$ and transepithelial potential depend strongly on the thermodynamic driving force driving the HCO_3^- pumping system and on the anion composition of the bath.

In the stimulated or activated state (last three col-

TABLE IV

Control of system parameters on fluid secretion in choline medium

See legend to Table III. In this medium, 44% of the Na^+ is replaced by choline (conditions as described in Table I). The control coefficients of $[Na^+]_b$ and $[Cl^-]_b$ are related to those of $[chol]_b$ and $[HCO_3^-]_b$, respectively, due to the constant sum of the medium cation and anion concentrations. The control coefficients of the modulated permeability coefficients, P_{Im}, are also given in this table. Noteworthy values are underlined.

Control coefficient (C) of	C with respect to				C with respect to			
	J_V	$[Na^+]_s$	$[Cl^-]_s$	ϕ	J_{Vm}	$[Na^+]_{sm}$	$[Cl^-]_{sm}$	ϕ_m
act	0.89	0.05; < 0	−0.21	0.10	0.79	0.11	−0.24	0.14
P_{Na^+}	0.04	0.01	0.04	−0.05	0.02	0.01	0.02	−0.02
P_{chol}	0.04	−0.06	0.04	−0.06	0.02	−0.02	0.02	−0.02
P_{Cl^-}	0.09	0.02	0.11	0.01	0.12	0.02	0.10	0.02
$P_{HCO_3^-}$	−0.07	> −0.01; < 0	0.02	−0.01	−0.04	−0.01	0.01	−0.01
α	0	0	0	0	0.10	−0.11	0.10	−0.11
Sum	1	0	0	0	1	0	0	0
K	0.90	0.06	−0.21	0.11	0.80	0.11	−0.25	0.15
$[Na^+]_b$	1.49	0.25	0.38	−0.66	1.19	0.65	0.06	−0.28
$[Cl^-]_b$	0.71	0.04	0.83	0.08	0.84	0.11	0.74	0.15
$P_{Na^+,m}$	0	0	0	0	0.01	< 0.01; > 0	0.01	−0.01
$P_{Cl^-,m}$	0	0	0	0	0.02	< 0.01; > 0	0.01	< 0.01; > 0
$P_{HCO_3,m}$	0	0	0	0	−0.02	−0.01	0.01	−0.01
$P_{chol,m}$	0	0	0	0	0.09	−0.11	0.07	−0.11

umns of Table III) most control coefficients are only slightly different from the ones in the unactivated state. The control of J_{Vm} by α is minor, as is the case for $[Cl^-]_{sm}$. The parameter ϕ is under slight negative control of α, which means that also the transepithelial electrical potential difference is decreased when α is increased.

In Table IV, the values of the control coefficients in the case of replacement of Na^+ by choline are shown. Compared to the values of Table III, the control coefficients are similar in magnitude, although differences do exist. The control coefficient of P_{chol} with respect to J_V is small (0.04), whereas, relative to the other coefficients, it is large with respect to $[Na^+]_s$ and ϕ. The factor act is also important in determining $[Na^+]_s$ and thus $[chol]_s$. The control coefficient of K with respect to J_V, $[Cl^-]_s$ and ϕ is again substantial, and the control by $[Na^+]_b$, i.e., by the Na^+ and choline concentrations in the bathing medium, of J_V, $[Na^+]_s$ and ϕ, and also of $[Cl^-]_s$ is considerable. The coefficient of $[Cl^-]_b$ is relatively large with respect to J_V and $[Cl^-]_s$, but small with respect to ϕ. In the activated state (cf. the four right-hand columns of Table IV) the values of the control coefficients are somewhat changed. The amount of stimulant added, which determines the magnitude of the parameter α, now has a relatively large effect on $[Na^+]_{sm}$, $[Cl^-]_{sm}$ and ϕ_m, but does not strongly affect J_{Vm}. Except for the choline permeability, P_{cholm}, the activated ion permeabilities are of minor significance in the control of the variables J_{vm}, $[Na^+]_{sm}$ and $[Cl^-]_{sm}$, and ϕ_m.

The inner workings of the model

One may summarize the model for pancreatic fluid secretion as: (1) a transcellular electrogenic HCO_3^- pump, (2) paracellular movement of Na^+, (3) transepithelial water flow to establish isotonicity, and (4) paracellular NaCl and $NaHCO_3$ flow allowing for additional water flow. Thus, the system would work as follows:

(i) The cells pump HCO_3^- across the epithelium, which tends to increase the osmolarity of the secreted fluid.

(ii) Because this pumping is electrogenic, a transepithelial electric potential difference, negative on the secretory side, is generated, which drags Na^+ through the paracellular pathway causing an additional tendency to increase the osmolarity of the secretion.

(iii) Due to (i) and (ii) water is pulled across the epithelium.

(iv) As a consequence of (iii) there will be a concentration gradient for Cl^- across the epithelium, which will drive Cl^- across and thus lead to an additional water flow. However, this flow may be inhibited by the electric potential generated by (i).

It follows that there are three components to the water flow. The first (J_{w,HCO_3}) is related to the active HCO_3^- flow and is independent of the development of an electric potential. The second ($J_{w,el}$), linked to the Na^+ flux, depends primarily on the generation of the potential. The third ($J_{w,Cl}$), linked to the Cl^- and ensuing Na^+ fluxes, depends on the permeability of the paracellular shunt for Cl^- and Na^+.

TABLE V

Results of simulation experiments in normal medium

The results were calculated by applying the model equations under the simulated conditions indicated in the left column. One parameter was set at a given value, while the other parameters were unchanged and had the values shown in Table II. Results are given for fluid secretion rate (J_V), Cl^- and Na^+ concentrations and ϕ, all under both normal and stimulated conditions. Underlined values are particularly noteworthy (see Results). ( ), not measurable variable.

	J_V	J_{Vm}	$[Cl^-]_s$	$[Cl^-]_{sm}$	$[Na^+]_s$	$[Na^+]_{sm}$	ϕ	ϕ_m
Standard	100	105	0.51	0.54	1	1	1.13	1.10
$P_{Cl^-} = 0$	47	61	0	0.23	1	1	1.06	1.06
$P_{Cl^-m} = 0$	100 [a]	101	0.51 [a]	0.53	1 [a]	1	1.13 [a]	1.10
$P_{HCO_3^-} = 0$	101	106	0.51	0.54	1	1	1.13	1.10
$P_{HCO_3^-m} = 0$	100 [a]	105	0.51 [a]	0.54	1 [a]	1	1.13 [a]	1.10
$P_{Na^+} = 0$	0	93	(0.04)	0.49	(1)	1	(20.2)	1.34
$K = 1$	0	0	(0.89)	(0.89)	(1)	(1)	(1.00)	(1.00)
$[Cl^-]_b = 0$	49	49	0	0	1	1	1.07	1.05
							$[HCO_3^-]_s$	$[HCO_3^-]_{sm}$
Ions → neutral molecules	91	97	0.58 [b]	0.59 [c]	0.89 [b]	0.91 [c]	0.53 [b]	0.50 [c]

[a] The same result as in the standard case.

[b] Sum = 0.58 + 0.89 + 0.53 = 2.

[c] Sum = 0.59 + 0.91 + 0.50 = 2.

TABLE VI

Results of simulation experiments in choline medium

See legend to Table V. In this medium, 44% of the Na^+ is replaced by choline (conditions as in Table I). ( ), not measurable variable.

	J_V	J_{Vm}	$[Cl^-]_s$	$[Cl^-]_{sm}$	$[Na^+]_s$	$[Na^+]_{sm}$	ϕ	ϕ_m
Standard	45	52	0.44	0.51	0.93	0.78	1.77	1.48
$P_{Cl^-} = 0$	24	35	0	0.30	0.89	0.74	1.65	1.38
$P_{Cl^-m} = 0$	45 [a]	51	0.44 [a]	0.50	0.93 [a]	0.78	1.77	1.47
$P_{HCO_3^-} = 0$	48	54	0.43	0.50	0.93	0.78	1.79	1.49
$P_{HCO_3^-m} = 0$	45 [a]	53	0.44 [a]	0.51	0.93 [a]	0.78	1.77	1.48
$P_{Na^+} = 0$	12	49	0.11	0.48	0	0.75	8.20	1.60
$P_{chol} = 0$	43	51	0.41	0.50	1	0.80	1.90	1.51
$P_{cholm} = 0$	45 [a]	45	0.44 [a]	0.45	0.93 [a]	0.93	1.77	1.75
$K = 1$	0	0	(0.89)	(0.89)	(0.56)	(0.56)	(1.00)	(1.00)
$[Cl^-]_s = 0$	26	26	0	0	0.89	0.71	1.66	1.31
							$[HCO_3^-]_s$	$[HCO_3^-]_{sm}$
Ions → neutral molecules	38	45	0.73	0.72	0.53	0.53	0.69	0.58

[a] The same result as in the standard case.

Using the final model as formulated above, we may now perform 'hypothetical' (Gedanken) experiments that would be difficult to perform on the actual biological system. For instance, with respect to the relevance of the transepithelial potential for fluid secretion, we can ask the question: what would be the water flow if there would be no electrical coupling between the ion fluxes. Therefore, we performed a computer experiment where we replaced HCO_3^-, Cl^-, Na^+ and K^+, and choline by neutral molecules (such as sugars) and then repeated the calculations retaining the permeability coefficients of the previously ionic, now neutral species as well as the activity parameters of the pump. In the equations this came down to setting ϕ equal to 1, i.e. setting $\Delta\psi$ to 0, and relaxing Eqn. 9 to:

$$[Na^-]_s + [\text{choline}]_s + [Cl^-]_s + [HCO_3^-]_s = 2 \qquad (11)$$

The bottom lines of Tables V and VI show the results of this calculation. It can be seen that in the absence of electric coupling, the water flow would only be 9% smaller than in the presence of this coupling (Table V). In the case of substituting choline for a large fraction of the Na^+ in the bath, the electric coupling is responsible for 16% (100(45 − 38)/45 percent) of the water flow (Table VI).

Other simulated experiments were those in which we examined the importance of the various ions for fluid secretion, by calculating the effect of setting the ion permeabilities to zero (Table V). Reducing P_{Cl^-} to zero reduced the volume flow, J_v, by 53%, decreased $[Cl^-]_s$ to 0 with no effect on $[Na^+]_s$ and slightly reduced ϕ. It should be noted that a decrease of ϕ always indicates a decrease in $|\Delta\psi|$, i.e., a smaller, lumen-negative transepithelial potential. A zero Cl^- permeability also amplified the stimulant-induced increase of J_V, from 5 to 30%. On the contrary, setting $P_{HCO_3^-}$ to zero had a slightly positive effect on J_V and J_{Vm}. Lowering P_{Na^+} to zero completely abolished fluid secretion, caused Cl^- to virtually disappear from the secreted fluid and ϕ to increase considerably, while it resulted in the relatively largest increase in J_V upon stimulation (from 0 to 93). For the unstimulated conditions, the latter experiments would be comparable to replacing Cl^-, HCO_3^- or Na^+ by ions with very low permeability coefficients. Finally, a zero P_{Cl^-m} strongly reduced $J_{Vm} - J_V$, whereas setting $P_{HCO_3^-m}$ to zero did not affect the stimulated fluid secretion at all. Setting act equal to zero, or K equal to 1 also abolished fluid secretion under both control and stimulated conditions, which means that a free energy input to the HCO_3^- pump and a non-zero activity of that pump (the latter not shown) is essential for fluid secretion. When $[Cl^-]_b$ was lowered to zero, i.e. Cl^- replaced by HCO_3^- (since $[Cl^-] + [HCO_3^-]$ = constant), fluid secretion was reduced by 51%, while the only anion in the secreted fluid was, obviously, HCO_3^- (Table V). Upon reducing $[Cl^-]_b$ to zero, stimulation did no longer result in an increase in J_V.

When we simulate the above-mentioned experiments in choline medium, the results are essentially similar or a little more pronounced (Table VI). Setting P_{Cl^-} to zero again caused a larger increase in J_V upon stimulation (from 24 to 35) than under normal conditions (from 45 to 52), while setting P_{Cl^-m} to zero now had little effect on the increase of J_V by CCK-8 stimulation. The effects of setting $P_{HCO_3^-}$ and $P_{HCO_3^-m}$ to zero were again negligible as is the case in normal medium. Re-

ducing P_{Na^+} to zero caused a large reduction in J_V, with a relatively large increase of J_V upon stimulation (from 12 to 49). In this case, $[Cl^-]_s$ was again considerably decreased, and ϕ increased from 1.77 to 8.20. When P_{chol} was set to zero, J_V was only very slightly decreased (from 45 to 43), and also J_{Vm} as well as the other parameters were barely affected. In other words, replacing Na^+ by a cation for which the epithelium is impermeable, has only a slightly more pronounced effect than replacement by choline. This can be understood from the fact that, compared to P_{Na^+}, P_{chol} is negligible or, very close to zero. Setting P_{cholm} to zero caused J_V, $[Cl^-]_s$, $[Na^+]_s$ and ϕ to remain at their control values upon stimulation. By setting act equal to zero, K to 1, or $[Cl^-]_b$ to zero, the same results were obtained as by this simulation in normal medium.

Discussion

In this paper, we present a quantitative elaboration for a qualitative scheme of fluid secretion from the isolated rabbit pancreas. This model accurately reproduces the results obtained in previous studies [7,8,22,25,26], which verifies the validity of the original scheme. Quantitative models have the advantage of forcing one to be explicit and precise. For instance, in our qualitative scheme, it was not specified to what extent the inhibition of fluid secretion in the case of Na^+ substitution is due to a reduction in the activity of the 'HCO_3^- pump', and to what extent it is due to inhibition of passive paracellular cation flow. In the case of K^+ substitution the fluid secretion rate, J_V, was linearly, i.e. proportionally, reduced upon decreasing $[Na^+]_b$ whereas $J_{HCO_3^-}$ was inhibited more than proportionally [6,8]. Upon substitution of Li^+, however, $J_{HCO_3^-}$ was inhibited linearly by the decrease in $[Na^+]_b$ [8].

These observations led us to specify the model in terms of a linear dependence of $J_{HCO_3^-}$, and not of J_V, on $[Na^+]_b$ in general, with an additional inhibitory effect of K^+ on the HCO_3^- pump. The actual mechanism, or site, at which these dependencies come into play could be the Na^+/HCO_3^--cotransport system operative in the basolateral membrane of the pancreatic fluid-secreting cell [25]. Inhibition of J_V by replacement of Na^+ by other cations would be partly due to inhibition of $J_{HCO_3^-}$ through reduction of $[Na^+]_b$ (for all substitutions) or due to a special inhibitory effect on the HCO_3^- pump (for K^+), and partly due to inhibition of passive cation permeation (for all substitutions).

The results predicted by this model are in very good agreement with the experimentally obtained results on both fluid secretion rate and secretory ion concentrations as well as transepithelial potential. Paradoxically, the flow of a particular ion can be reduced while its concentration in the secreted fluid is increased. For example, the removal of Na^+ causes in all cases an increase of $[Cl^-]_s$, whereas the Cl^- flux (J_{Cl}) is decreased (Table I). Here the model helped us to further understand the system. It allowed us to verify that the latter is the result of (i) a decreased water flux due to decreased pumping of HCO_3^-, and therefore of cations, water and thus anions, and also of (ii) an effect of Na^+ substitution on $\Delta\psi$, leading to a decreased passive J_{Cl} in the case of Li^+ and choline substitution, and a slightly increased J_{Cl} in the case of K^+ substitution. The net result of this is that Na^+ substitution has an inhibitory effect on both J_{HCO_3} and J_{Cl} but tends to increase $[Cl^-]_s$ because J_{Cl} is less inhibited than $J_{HCO_3^-}$.

The effect of stimulation by CCK-8 is satisfactorily explained by a differential increase in the shunt permeability of all cations and anions. The permeability increase can be visualized as the opening of a separate ion channel with relative ion permeabilities which are different from those of the normal channel (Table II). The model assumed for both channels a much larger permeability for cations than for anions.

Quantitative models also allow the evaluation of control coefficients (Tables III and IV). In this case, these suggest that fluid secretion rate is predominantly under control of the pump activity and to some extent also of the Cl^- permeability, in both normal medium and choline medium. The equilibrium constant K, i.e. the metabolic free energy driving the HCO_3^- pump, and $[Na^+]_b$ and $[Cl^-]_b$ all have a large regulatory potential.

As pointed out above, the dependence of fluid and ion secretion on $[Na^+]_b$ is two-fold, through pump and shunt, and this explains that the control coefficient of $[Na^+]_b$ can be larger than 1. This control coefficient is in part determined by the Na^+ dependence, or the so-called elasticity coefficient of the pump and the shunt with respect to Na^+ [9,12,23]. In the choline medium, these two elasticity coefficients are 1 and 15.9, which is in agreement with the result obtained of a control coefficient of $[Na^+]_b$ on J_V of 1.49 (Table IV), since the shunt permeability for Na^+ has a approx. 25-times lower control coefficient on J_V than the pump activity ($1.49 = 0.893 \cdot 1 + 0.035 \cdot 15.9$).

Quantitative models allow one to simulate experiments that have not been carried out yet. This can be useful in terms of increasing the understanding of how the model, and supposedly the system, works. From the results of such simulations we infer that in normal and choline medium the Cl^- and Na^+ permeability, but not the HCO_3^- permeability are essential for fluid secretion, and that a positive free energy difference of the pump ($\Delta G > 0$, $K \gg 1$) is a necessary condition for secretion. If we set P_{Cl^-} at 0, we obtain a theoretical result of 53% inhibition of J_V. The experimental equivalent of this simulation, which is the replacement of Cl^- by a largely impermeant anion such as isethionate, led to approx. 50% reduction of fluid secretion rate [7]. The activated permeabilities for Cl^- (P_{Cl^-m}) and Na^+ (P_{Na^+m}) are

important, whereas $P_{HCO_3;m}$ is not important for the stimulated fluid secretion rate, J_{Vm}.

An implication of the simulation of the absence of electric coupling (Tables V and VI, bottom line) would be that if one would succeed in short circuiting the pancreatic epithelium, this would only lead to a minor reduction in volume flow. Thus, mainly osmotic forces are responsible for ion and therefore water secretion in the pancreas.

The HCO_3^- transporting mechanism in the pancreas is apparently located in the ductular and/or centroacinar cells. However, previous biochemical and histochemical studies, and the fact that CCK and acetylcholine receptors are located exclusively on acinar cells, have indicated that paracellular transport is an acinar event [26]. According to this concept, HCO_3^- ions would be transported actively through the ductular cell, and cations and anions would be transported passively through the junctions between acinar cells. Where water is secreted remains to be answered. Although our model cannot provide the answer, it does not depend on it either.

Our model for fluid secretion hinges on some basic assumptions. Some of them can be readily verified, others are subject to questioning. However, with a few simple underlying hypotheses, the model is able to simulate the experimental results described and discussed in previous papers [6–8] with remarkable accuracy. Therefore, it is powerful enough to give a possible explanation for these results. The complexity of the secretory system, however, which might well be greater than as proposed in our model, could account for small differences between model and reality.

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