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Role of monovalent cations in fluid secretion from the exocrine rabbit pancreas

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The role of Na^+ in fluid secretion by the isolated rabbit pancreas was investigated. The fluid secretion rate is reduced upon replacement of Na^+ in the bathing medium by Li^+, K^+ or choline. The inhibition depends on the nature of the substituting cation, and is largest with choline. Upon replacement, the substituent cation appears in the secreted fluid, and the Na^+ concentration in the secreted fluid is decreased in a mirror-like fashion. When Na^+ is replaced by Li^+ or choline, the secretory Na^+ concentration is decreased, although less than in the bathing medium, and the K^+ concentration is increased. When Na^+ is replaced by K^+, the Na^+ and the K^+ concentration in the secreted fluid are approximately equal to their bathing medium concentrations. In the Li^+ and choline medium, stimulation of the pancreas by carbachol or CCK-8 increases the fluid secretion rate. In addition, it increases the Li^+ or choline concentration, and decreases the Na^+ and K^+ concentrations in the secreted fluid. In normal and K^+ medium, stimulation causes only a slight increase in fluid secretion rate, with no change in the secretory Na^+ concentration. In normal medium, stimulation leads to a decrease in the secretory K^+ concentration. The effects of replacing Na^+ appear to be the result of a direct inhibition of the active HCO_3^- transport underlying secretion, and an indirect inhibition related to the permeability of the pancreas for the various cations. The stimulants are likely to act by increasing the permeability of the tight junctions.

Introduction

The isolated rabbit pancreas spontaneously secretes a fluid with a high concentration of HCO_3^-. Fluid secretion can be stimulated by secretin, but not by acetylcholine or CCK. The fluid secretion process appears to be based on a Na^+-gradient dependent transport of HCO_3^- through the cell [1–4]. The cations Na^+ and K^+ are thought to be transported via a paracellular route [5], while Cl^- enters the secretory lumen through either a transcellular or a paracellular pathway [3].

Passive, paracellular permeation of charged and uncharged molecules has been well documented for the rabbit pancreas [6]. The permeability for a given molecule that is passively transported through the epithelium can be described by an apparent reflection coefficient or by the ratio of its medium and secretory concentration [7,8]. Thus, Na^+ and K^+ appear to be freely and equally permeating, and the apparent reflection coefficients for NaCl and KCl are 0.50 and 0.51, respectively [7]. In a normal incubation medium with Na^+ as the main cation, the concentrations of Na^+ and K^+ in the secreted fluid are virtually equal to those in the bathing medium [5,9]. In contrast, choline and the divalent cations Ca^{2+} and Mg^{2+} appear in the secreted fluid in concentrations that are much lower than those in the bathing medium [1,5]. The uncharged molecules sucrose, lactose, mannitol and inulin, and horse radish peroxidase (HRP) also permeate the epithelium at rates decreasing in this order [6,10].

The paracellular permeability can be increased by stimulants of the enzyme secretion process such as acetylcholine and the C-terminal octapeptide of chole-

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Abbreviations: CCK, cholecystokinin; CCK-8, cholecystokinin-octapeptide.

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cystokinin (CCK-8), as determined for Ca^{2+} and Mg^{2+}, and for sucrose, HRP and other uncharged molecules [6,7,10,11]. This permeability increase can be specifically inhibited by low concentrations of receptor antagonists [6,12]. The paracellular passage of HRP in the rabbit pancreas is mainly governed by the acinar cell junctions, since the permeability of these junctions is modulated by the stimulants [10]. Similar findings on tight junctional HRP permeability have been obtained in the submandibular gland of the dog and the rabbit and in the parotid gland of the rat [13–15].

From the effects of the replacement of Na^+ by K^+, choline or sucrose it has previously been suggested that the paracellular permeability for the secretory ion is a determining factor for the rate of fluid secretion [5]. Therefore, we now investigate the effects of Na^+ replacement by various cations on fluid secretion rate and composition, and the effects of stimulants on ion and fluid secretion under the different conditions, and relate these effects to the Na^+ dependence of the fluid secretion process and the permeability for the various cations tested. Our discussion and conclusions are based on the conceptual model of secretion described in previous papers [4–7]. This model will be worked out mathematically in the subsequent paper [23].

Materials and Methods

Preparation and incubation

Male and female New Zealand white rabbits of 3–4 kg are used. The animals are killed by a blow on the neck, immediately followed by carotic exsanguination. The pancreas is prepared essentially as described by Rothman [16] and modified by us [17]. The isolated pancreas is mounted on a frame and incubated in a bath containing 350 ml bathing medium. The main pancreatic duct is cannulated and the secreted fluid is collected. The pancreas is preincubated for 1 hour in a balanced Krebs-Ringer bicarbonate medium in order to reach a steady-state condition. The composition of the medium is (in mmol/l): Na^+ 144, K^+ 4.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 131, HCO_3^- 25, $H_2PO_4^-/HPO_4^{2-}$ 1.2, glucose 5.5 (pH 7.4). The medium is continuously gassed with carbogene (95% O_2, 5% CO_2). After the preincubation period, the medium is replaced by fresh medium and the experiment is started. The secreted fluid is collected in preweighed plastic tubes and from each fraction samples are taken for the appropriate assays.

The first hour of incubation (control period) is carried out in normal Krebs-Ringer medium. This is generally followed by two experimental periods of 60 min each. In the first experimental period the normal medium is replaced by a medium in which Na^+ is partially replaced by Li^+, K^+ or choline and in the second experimental period carbachol or CCK-8 is added. The secretory rate and composition of the secreted fluid reach a steady-state level within 30–60 minutes of the first experimental period. For calculations the mean values are taken of the final 20-min intervals of the control period and the first experimental period, and of the 10–40-min interval after stimulation in the second experimental period. In the experiments with radioactive tracers, [^{3}H]sucrose (0.01 μM, 60 mCi/mol) or [^{14}C]choline (2.8 μM, 1.5 mCi/mol) (RCA, Amersham, U.K.) are added to the bathing medium. In addition, 2 mM cold sucrose or choline are added in the control and experimental periods.

The apparent reflection coefficients for NaCl, KCl, LiCl and choline chloride are calculated as the ratio of the percentage inhibition of the fluid secretion rate by 100 mosM NaCl, KCl, LiCl or choline chloride and the percentage inhibition by 100 mM sucrose, assuming a reflection coefficient of 1.0 for sucrose [7].

The statistical test applied was the *t*-test of Student for the difference between two means, for independent samples or dependent samples.

Determinations

The volume of the secreted fluid fractions is determined by weighing the collecting tubes on an automatic Mettler electronic balance, assuming a fluid density of 1.0. Samples of 5–15 μl bathing medium or secreted fluid are diluted with distilled water to 3 ml. Na^+ and K^+ in these dilute samples are measured in an Eppendorf flame spectrophotometer and Li^+ is measured by atomic absorption spectrophotometry. Calibration curves for all three cations are virtually linear.

The choline concentration of the secreted fluid is calculated from the difference between the ($Na^+ + K^+$) concentration in the normal medium of the preceding control period and the ($Na^+ + K^+$) concentration in the choline medium. The Cl^- concentration of the bathing medium or secreted fluid is measured coulometrically by titration in an Aminco-Cotlove chloride titrator, and the HCO_3^- concentration is calculated assuming that the ($Na^+ + K^+$ + cation) concentration is equal to the ($Cl^- + HCO_3^-$) concentration.

Results

Effects of replacement of Na^+ by Li^+

Replacement of Na^+ by Li^+ reduces the fluid secretion rate of the isolated rabbit pancreas (Fig. 1). Upon the replacement of approx. 119 mM Na^+ by 119 mM Li^+, the Na^+ concentration in the secreted fluid decreases to a value which is slightly higher than its concentration in the bathing medium, and Li^+ appears in the secreted fluid in a concentration which is slightly lower than its bathing medium concentration. In addition, the K^+ concentration in the secreted fluid is increased (Fig. 1, Table I), and the Cl^- concentration in the secreted fluid is also increased (Table I). Fig. 2

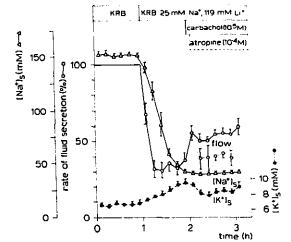

Fig. 1. Effect of replacement of Na^+ by Li^+ and subsequent carbachol stimulation on the rate of fluid secretion (○), and the concentrations of Na^+ (△) and K^+ (●) in the secreted fluid of the isolated rabbit pancreas. The dotted line indicates the rate of fluid secretion when atropine (10^{-4} M) is given 5 min after addition of carbachol (10^{-5} M). Values are means with S.E. of five experiments.

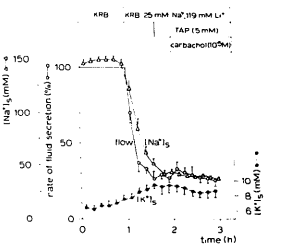

Fig. 3. Effect of 2,4,6-triaminopyrimidine (TAP) and subsequent carbachol stimulation on the rate of fluid secretion (○) and on the concentrations of Na^+ (△) and K^+ (●) in the secreted fluid of the isolated rabbit pancreas. Values are means with S.E. of four experiments.

shows that the fluid secretion rate is linearly decreased with the concentration of Na^+ in the bathing medium, and that the concentrations of Na^+ and Li^+ in the secreted fluid are also linearly related to the bathing medium concentration of Na^+.

In normal Krebs-Ringer medium, stimulation of the pancreas with carbachol (10^{-5} M) markedly increases protein secretion [16]. In addition, the K^+ concentration of the secreted fluid decreases from 6.8 to 6.1 mM (Table I). The fluid secretion rate is only slightly increased with 4%. However, when Na^+ is replaced by Li^+ (approx. 119 mM), carbachol markedly increases fluid secretion immediately after its addition (Fig. 1, Table I). Concurrently, the K^+ and Na^+ concentrations in the secreted fluid decrease, while the Li^+ concentration is slightly elevated (Table I). The increase in protein output is the same as in normal medium (results not shown). Stimulation with CCK-8 (10^{-8} M) in the Li^+ medium has the same effects on fluid secretion rate and ion concentrations in the secreted fluid as stimulation with carbachol (10^{-5} M) (Table I). When only half of the Na^+ in the bathing medium is replaced by Li^+ (approx. 77 mM), only the effect of carbachol stimulation on the K^+ concentration is obvious (Table I).

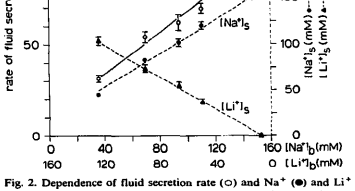

Fig. 2. Dependence of fluid secretion rate (○) and Na^+ (●) and Li^+ (▲) concentrations in the secreted fluid on the Na^+ and Li^+ concentrations in the bathing medium of the isolated rabbit pancreas. The results on fluid secretion rate are expressed as percentage of the secretion rate in normal Krebs-Ringer medium. Results are given as means with S.E. for four or five experiments.

From previous studies, we know that 2,4,6-triaminopyrimidine (TAP) inhibits the carbachol-induced increase of the paracellular permeability for small non-electrolytes and divalent cations [18]. In the medium with 119 mM of the Na^+ replaced by Li^+, addition of TAP ($5 \cdot 10^{-3}$ M) clearly inhibits the effect of carbachol (10^{-5} M) on the fluid secretion rate and on the decrease of the K^+ concentration in the secreted fluid, but not the effect on protein secretion. TAP by itself has no effect on either one of these parameters (Fig. 3, Table I).

It has been found previously that the cholinergic antagonist atropine also inhibits the increase of the paracellular sucrose permeation due to carbachol stimulation, even when it is added up to 10 min after carbachol [6]. When atropine (10^{-4} M) is added to the medium with 119 mM Li^+ 5 min after addition of carbachol (10^{-5} M) it reverses the increase in fluid secretion rate and inhibits the decrease of the K^+ concentration (Fig. 1, Table I).

Omission of Ca^{2+} from the medium containing 119 mM Li^+ has no effect on the stimulation of the fluid secretion rate and the decrease of the K^+ concentration in the secreted fluid by carbachol (10^{-5} M) (Table I).

TABLE I

Effects of Na^+-substitution and stimulation on fluid secretion in the isolated rabbit pancreas

The pancreas is incubated for successive 60-min periods in normal medium (control period), medium with Na^+ replaced by another cation (first experimental period), and in the latter medium with the stimulant carbachol (10^{-5} M) or CCK-8 (10^{-8} M) added (stimulation period). The ionic composition of the media and the presence of stimulant are indicated, and the results on flow rate and composition of the secreted fluid are given in *italics* in the line(s) below. Values are means with S.E. from the final 20-min intervals of the control and first experimental period, and from the 10–40-min interval of the stimulation period. *N*, number of experiments.

Medium	addition *flow rate*	$[Na^+]_b$ $[Na^+]_s$	$[K^+]_b$ $[K^+]_s$	$[Li^+]_b$ $[Li^+]_s$	$[chol]_b$ $[chol]_s$	$[Cl^-]_b$ $[Cl^-]_s$	*N* *N*
Control	–	146±2	5.3±0.1	–	–	135±1	17
	carb	147±2	6.7±0.2	–	–	135±1	7
	100	*148±2*	*6.8±0.2*	–	–	*72±1*	
	104±11	*146±4*	*6.1±0.3*[c]	–	–	*81±4*[b]	
Lithium	–	67±2	5.7±0.2	74±2	–	139±5	6
	carb	67±2	6.1±0.2	74±2	–	139±5	6
	54± 7[a]	*78±1*[a]	*8.6±0.3*[a]	*73±2*	–	*83±7*[a]	
	61± 8	*75±2*	*7.4±0.4*[c]	*81±2*	–	*97±8*[c]	
	–	34±2	7.5±0.2	105±4	–	138±2	6
	CCK-8	36±2	8.7±0.3	103±4	–	138±2	6
	35± 3[a]	*45±1*[a]	*11.1±0.4*[a]	*103±2*[a]	–	*94±4*[a]	
	49± 5[c]	*36±3*[c]	*9.0±0.9*[c]	*109±1*[c]	–	*103±5*[c]	
	–	38±1	6.4±0.2	107±2	–	138±1	17
	35± 2[a]	*45±1*[a]	*9.1±0.3*[a]	*105±3*[a]	–	*96±2*[a]	
	carb	41±2	7.4±0.2	107±4	–	138±2	5
	52± 2[b]	*39±2*[b]	*7.9±0.3*[c]	*108±5*	–	*95±5*	
	carb-TAP	41±3	7.4±0.3	107±5	–	138±2	4
	36± 4	*42±2*	*8.5±0.6*	*107±5*	–	–	
	carb+atro	41±3	7.4±0.3	–	–	–	3
	41± 7	*41±2*	*8.5±0.3*	–	–	–	
	carb-Ca^{2+}	41±3	7.4±0.3	–	–	–	3
	59± 9[b]	–	*7.6±0.4*[c]	–	–	–	
Choline	–	77±2	5.2±0.3	–	66±2	140±2	4
	CCK-8	81±2	6.5±0.3	–	62±2	140±2	4
	45± 4[a]	*128±4*[a]	*10.5±0.2*[a]	–	*12±3*[a]	*77±3*	
	52± 5	*104±2*[c]	*9.5±0.4*[c]	–	*39±3*[c]	*81±4*[c]	
	–	38±2	–	–	107±3	–	3
	CCK-8	38±2	–	–	107±3	–	3
	13± 2[a]	–	–	–	*66±8*[a]	–	
	17± 3[c]	–	–	–	87±2[c]	–	
K^+	–	102±2	57 ±3	–	–	142±2	4
	69± 5[a]	*115±3*[a]	*60 ±2*[a]	–	–	*99±6*[a]	
	–	54±1	99 ±4	–	–	142±2	6
	CCK-8	55±2	98 ±5	–	–	143±3	5
	40± 4[a]	*61±1*[a]	*97 ±7*[a]	–	–	*119±4*[a]	
	43± 5	*57±1*[b]	*95 ±4*	–	–	*118±4*	
Ouabain	–	148±5	8.0±0.3	–	–	–	3
	carb	146±5	9.9±0.4	–	–	–	3
	28± 9[a]	*145±3*	*10.3±0.4*[a]	–	–	–	
	29± 5	*141±3*	*9.1±0.5*	–	–	–	

[a] Significantly different from previous control period with $P < 0.05$ (*t*-test Student).

[b,c] Significantly different from previous experimental period without stimulus with $P < 0.05$ (*t*-test Student for independent ([b]) or dependent ([c]) samples).

Effects of replacement of Na^+ by choline

Replacement of Na^+ by choline decreases the fluid secretion rate as shown in Fig. 4. The reduction in fluid secretion rate in the case of choline substitution is more pronounced than in the case of Li^+ substitution, e.g. replacement of 74% of the Na^+ in the medium leads to a significantly larger decrease of fluid secretion rate (87%) when Na^+ is replaced by choline than when it is replaced by Li^+ (65%) (Table I). Upon the replacement of Na^+ by choline, e.g. in the case of replacement of 47% of the Na^+, the Na^+ concentration in the secreted fluid becomes much higher than in the bathing medium (128 mM vs. 77 mM), and choline appears in the secreted fluid in a concentration of only 18% of its bathing medium concentration (12 mM vs. 66 mM). In addition, the K^+ concentration in the secreted fluid is strongly increased (Fig. 4) and the Cl^- concentration is slightly increased (Table I).

In the experiments in which Na^+ is substituted by choline, atropine (10^{-4} M) is always added to the media in order to inhibit cholinergic stimulation by choline. Addition of CCK-8 (10^{-8} M) to the medium containing 77 mM Na^+ and 66 mM choline stimulates enzyme secretion and increases the fluid secretion rate by 16% (Fig. 4). The Na^+ and K^+ concentrations in the secreted fluid decrease, while the choline concentration increases by an equivalent amount. The Cl^- concentration in the secreted fluid slightly increases (Table I). Addition of TAP to the choline medium does not alter the effect of CCK-8 on fluid secretion rate and secretory ion concentrations (results not shown).

When 119 mM Na^+ is replaced by 119 mM choline, the fluid secretion rate is decreased by 87%, and choline appears in the secreted fluid in a concentration of 62% of its bathing medium concentration (66 mM vs. 107 mM). In this case, CCK-8 (10^{-8} M) increases the fluid

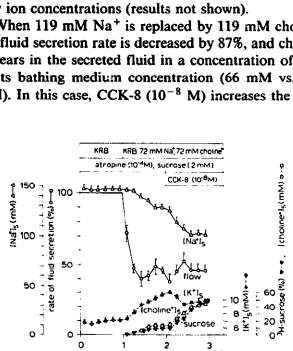

Fig. 4. Effect of replacement of Na^+ by choline and effect of stimulation by cholecystokinin-octapeptide (CCK-8) in choline medium on the rate of fluid secretion (○) and on the concentrations of Na^+ (△), K^+ (●), choline (□) and sucrose (▼) in the secreted fluid of the isolated rabbit pancreas. Values are means with S.E. of four experiments.

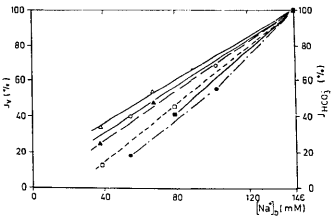

Fig. 5. Relation between the Na^+ concentration in the bathing medium and the fluid secretion rate or volume flow (J_V) in the isolated pancreas in the case of replacement of Na^+ by Li^+ (△), K^+ (○) or choline (□), and relation between the Na^+ concentration in the bathing medium and the flow of HCO_3^- ($J_{HCO_3^-}$) in the case of replacement of Na^+ by Li^+ (▲), K^+ (●) or choline (■). Results are calculated from means of 3–6 experiments, as given in Table I.

secretion rate by 31% and increases the choline concentration in the secreted fluid (Table I).

In Fig. 5, the effects of replacement of Na^+ by either Li^+, K^+ or choline on the rate of fluid secretion, i.e. volume flow (J_V), and on the rate of HCO_3^- secretion ($J_{HCO_3^-}$) are summarized. $J_{HCO_3^-}$ was calculated from the HCO_3^- concentration in the secreted fluid and the fluid secretion rate. $J_{HCO_3^-}$ was chosen as the plotted parameter because (1) fluid secretion in the pancreas is primarily dependent on the pumping of HCO_3^- [2], and (2) for the purpose of discussion.

Effects of replacement of Na^+ by K^+

Replacement of Na^+ by K^+ leads to inhibition of the fluid secretion rate. K^+ appears in the secreted fluid in approximately the same concentration as it is present in the bathing medium, and the secretory Na^+ concentration is also equal to its bathing medium concentration. As compared to normal medium, the Cl^- concentration in the secreted fluid is clearly increased (Table I). When the pancreas is stimulated by CCK-8 in K^+ medium, the fluid secretion rate is virtually unchanged, and the Na^+, K^+ concentrations in the secreted fluid remain unaltered (Table I).

Effects of ouabain

Since the replacement of Na^+ leads to a decrease of the Na^+ gradient across the cell membrane, we have also studied the effects of decreasing this gradient by the Na^+/K^+-ATPase inhibitor ouabain. Ouabain ($5 \cdot 10^{-6}$ M) inhibits the fluid secretion rate by 72%, increases the K^+ concentration and has no effect on the Na^+ concentration in the secreted fluid (Table I). When carbachol is added to the medium 60 min after ouabain addition the fluid secretion rate remains unchanged, the K^+ concentration in the secreted fluid is decreased and

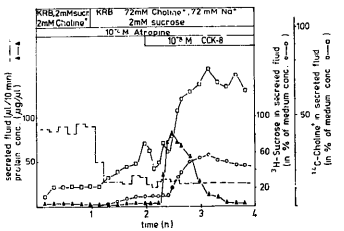

Fig. 6. Effect of CCK-8 stimulation in choline medium on the rate of fluid secretion (- - - - -), the protein concentration (▲), and the concentrations of sucrose (○) and choline (□) in the secreted fluid of the isolated pancreas. Results are typical for three experiments.

the protein secretion is stimulated although less than under normal conditions (Table I).

Effects of stimulation on paracellular permeability

We have investigated whether the effects of the stimulants in the low-Na^+ media are related to the previously found increase in paracellular permeability upon stimulation. Therefore, the effect of CCK-8 on the permeability of [^{3}H]sucrose and [^{14}C]choline is studied in the replacement media. Also, the apparent reflection coefficients for LiCl and choline chloride are determined in the absence and the presence of carbachol or CCK-8.

In the Li^+- and choline media with 2 mM [^{3}H]- or [^{14}C]sucrose and 2 mM [^{14}C]choline added, CCK-8 increases the secretion of choline and sucrose in a parallel and equivalent way (Fig. 6). The extent of the increase is about the same as in normal medium (Table II). When the choline concentration in the secreted fluid is calculated from the [^{14}C]choline ratio in the secreted fluid and bathing medium, and is compared with the concentration obtained by calculation from the deficit in cation concentration, we obtain nearly the same results (Table I and II). The apparent reflection coefficient for Li^+ is significantly decreased by stimulation with carbachol (10^{-5} M) from 0.67 ± 0.06 to 0.59 ± 0.10 (averages ± S.E. of six experiments, $P < 0.01$), whereas the reflection coefficient for choline (0.84 ± 0.08) is not significantly decreased by 10^{-8} M CCK-8 to 0.82 ± 0.10 (averages ± S.E. of five experiments).

Discussion

Fluid secretion in the exocrine rabbit pancreas is mediated by a Na^+-dependent anion/H^+ transport mechanism in the basolateral membrane of the ductular cell, which is driven by the Na^+ gradient generated by the Na^+/K^+-ATPase [2–5]. This transporter accumulates HCO_3^- in the cell which, in conjunction with the inside-negative membrane potential of the apical membrane, causes an electrochemical potential difference for HCO_3^- across the apical cell membrane and drives the exist of HCO_3^- through this membrane into the secreted fluid. Passive secretion of permeant cations is thought to follow the electrogenic secretion of HCO_3^-, which in turn drives the isotonic transport of water and thus of fluid through the epithelium. The ensuing concentration gradient of additional anions drives more salt through the epithelium further facilitating water flow

TABLE II

Effect of CCK-8 on the permeation of sucrose and choline in various media bathing the isolated rabbit pancreas

The pancreas is incubated in successive 60-min periods in normal KRB medium, Li^+ or choline medium, and the latter medium with CCK-8 (10^{-8} M) added as a stimulant. 2 mM [^{3}H]sucrose and 2 mM [^{14}C]choline are added to the media, and the permeation of these substances is expressed as the ratio of the radioactivity in the secreted fluid and the bathing medium (% of medium concentration), or the relative flow of sucrose or choline (J_{rel}), which is the rate of fluid secretion multiplied by the percentage of the medium concentration of the substance in the secreted fluid. Values are means with S.E., and N is the number of experiments.

Bathing medium	Addition	Sucrose		Choline			N
		% of medium concentration	J_{rel} (%)	% of medium concentration	J_{rel} (%)	mM	
Normal KRB medium	sucrose	6 ± 1	100	–	–	–	5
	+ CCK-8	23 ± 6	407	–	–	–	5
Lithium medium [a]	sucrose + choline	18 ± 2	108	23 ± 3	138	0.5 ± 0.1	3
	+ CCK-8	34 ± 1	282	44 ± 1	385	0.9 ± 0.1	3
Choline medium [b]	sucrose	9 ± 2	69	28 ± 2	214	20 ± 1	3
	+ CCK-8	37 ± 9	326	54 ± 9	479	39 ± 1	3

[a] Normal KRB with 119 mM Na^+ replaced by 119 mM Li^+.
[b] Normal KRB with 72 mM Na^+ replaced by 72 mM choline.

[23]. Apparently, the Na^+ ion is important in two distinct processes:
(1) the transepithelial transport of HCO_3^-, and
(2) the secretion of the most abundant permeant cation, i.e. the Na^+ ion itself.
Thus, the inhibitory effect of replacing Na^+ by another cation presumably is due to the fact that this ion cannot effectively substitute for Na^+ in either one or both of these processes.

Effect of Na^+-replacement

Inhibition of secretion due to the replacement of Na^+ by Li^+ has previously been found in the perfused cat pancreas [19] and the isolated rabbit pancreas [20]. Inhibition of fluid secretion when Na^+ is replaced by choline or NaCl by sucrose has also been observed in previous studies on the isolated rabbit pancreas [1,5] and perfused cat pancreas [9]. If the permeability of the epithelium for the replacing cation is lower than for Na^+, this might lead to impaired passive cation permeation. The apparent reflection coefficients for LiCl and choline chloride, which are higher than for NaCl, suggest that the permeabilities for Li^+ and choline are lower than for Na^+.

We suggest that this difference in ion permeabilities could, at least partly, lead to the decrease of the fluid secretion by Li^+ and choline. Also, the increase of the K^+ concentration in the secreted fluid in Li^+ or choline medium can thus be explained by the preferential secretion of K^+ over Li^+ or choline due to the larger, probably paracellular, permeability of K^+. However, the reflection coefficients determined as described in Materials and Methods are apparent in the sense that they are measured as the effect of the cations on fluid secretion rate itself. Therefore, they include potential direct effects of the ion substitutions on the fluid secretion rate, and can not be used unequivocally to explain an inhibitory effect on this very parameter. Nevertheless, other studies have shown that in various leaky epithelia the relative permeabilities of Na^+, K^+ and Li^+ increase in the order $P_{Li^+} < P_{Na^+} < P_{K^+}$ [21], which is in agreement with the relative values of our apparent reflection coefficients (this study, and Ref. 7). This permeability order suggests that the hydrated shell radius of a cation determines its permeability, which may indicate that the cations move through hydrophilic pores in the membrane, i.e. the tight junctions which constitute the paracellular permeation route.

In the media containing a cation with a lower permeability than Na^+, one would expect the transepithelial potential to be increased as compared to normal medium. This is indeed the case, if we consider K_s^+/K_b^+ as a measure for this potential (Table I).

In the case of replacement of Na^+ by K^+, the above mentioned type of inhibition is unlikely since the permeability for K^+ is approximately equal to or slightly higher than that for Na^+. However, the fluid secretion rate is strongly inhibited by this replacement. Therefore, the removal of Na^+ or the presence of a high concentration of K^+ or both inhibit fluid secretion in some other way. Inhibition of the fluid secretion process by Na^+ removal might occur at the level of the Na^+-dependent HCO_3^- transport step, which involves the transport of HCO_3^- against its electrochemical gradient from the bathing medium through the cell into the secretory lumen. The quantitative relationship between the Na^+ concentration in the bathing medium and the rate of this Na^+-dependent HCO_3^- transport is unknown. It could be either linear or non-linear, and could depend on the nature of the substituent cation.

A direct dependence of fluid secretion on Na^+ would, however, also be observed in the case of replacement of Na^+ by Li^+ or choline, and would add to an effect on passive permeability. Comparing the data for the various replacements quantitatively (Fig. 5) shows, surprisingly, that the inhibition of fluid secretion in the Li^+ case is less that in the K^+ case. Therefore, assuming that the above mentioned hypotheses about the two types of inhibition are correct, we have to propose a third, inhibitory effect of K^+ or stimulatory effect of Li^+ on fluid secretion. The more than proportional inhibition of the HCO_3^- flow upon replacement of Na^+ by K^+ and the proportional inhibition of this flow in the case of replacing Na^+ by Li^+ (Fig. 5) suggests a linear relationship between the rate of HCO_3^- pumping and Na^+ in the bathing medium, in combination with a specific inhibitory effect of K^+. In the subsequent paper we will work out a model in which the pumping of HCO_3^- is linearly dependent on Na^+ in the bathing medium, but is also competitively inhibited by K^+. Using this quantitative model, we will investigate whether the effects observed in this study can be explained solely by the limiting paracellular shunt permeability or HCO_3^--pump inhibition, or by the fact that we indeed are dealing with two or more types of inhibition.

The increase of the Cl^- concentration in the secreted fluid in the Li^+ and choline media might reflect a relatively increased extent of HCO_3^-/Cl^- exchange in the efferent ducts at the lower secretory rates. The stronger increase of the Cl^- concentration in the secreted fluid in the K^+ media is probably related to the depolarizing effect of K^+, which increases the Cl^- flow and therefore the ratio Cl_s^-/Cl_b^-, Cl^- being a permeant anion [2]. However, as we show in the accompanying paper the anion concentration profile of the secreted fluid is the complex result of all ion flow rates, and the increased Cl^- concentration in the cases of Li^+ and choline substitution may be due to the inhibition of the HCO_3^- pumping rate and the resulting reduction in water flow.

Effect of stimulation

Stimulation of the pancreas with CCK-8 or carbachol leads to an increase of the fluid secretion rate and to decreases in the concentrations of the more permeant cations concomitant with increases in the concentrations of the less permeant ions. In all cases, except in K^+ medium, the Cl^- concentration in the secreted fluid is increased, and the HCO_3^- concentration is decreased. In normal medium, the permeability for the cations Na^+ and K^+ is approximately equal, the transepithelial electrical potential difference is small and the increase in fluid secretion rate is very small. In the media in which Li^+ or choline are substituted for Na^+, the increase in fluid secretion rate upon stimulation is considerable, and is more pronounced when more of the Na^+ in the bathing medium is replaced by the substituent ion. In the experiments in which the bathing medium contains 78 mM Na^+ and 66 mM choline, the increase of the choline concentration and the decrease of both the Na^+ and K^+ concentrations upon stimulation occur in parallel with the increase of the sucrose concentration, and thus are likely to be the result of an increased passive, paracellular transport of choline at the expense of the transport of Na^+ and K^+.

If we assume that also in the Li^+ media an increased paracellular permeability for cations is responsible for the changes in the fluid secretion rate and the cation concentrations in the secreted fluid, then these changes should be accompanied by a decrease of the apparent reflection coefficient for LiCl, and they should be inhibitable by TAP, which has been shown to be a specific inhibitor of the carbachol-induced increase in paracellular permeability [18]. These two phenomena are indeed observed (Table I, Figs. 1 and 2).

The effect of CCK-8 in K^+ medium is minimal in the sense that the Na^+, K^+ and Cl^- and HCO_3^- concentrations are not affected. Only the fluid secretion rate is slightly but not significantly increased. This result can be understood from the fact that the permeabilities for Na^+ and K^+ are similar and in this case not flux-controlling [23].

The insensitivity of the increase of the fluid secretion rate to the presence of Ca^{2+} in the bathing medium suggests that this process is different from the increase of the acinar fluid secretion component upon stimulation by cholinergic or CCK-8-like agonists as observed in the rat [22].

In conclusion, an increase in paracellular permeability leads to an increased fluid secretion rate only to the extent that the fluid secretion rate was limited by slow paracellular permeation of the main cation. In normal medium, paracellular transport of Na^+ and K^+ appears to be virtually no limiting factor for fluid secretion. The increase in permeability by stimulatory agonists is probably an increase in permeability of the tight junctions for the various ions.

In the next paper, we will investigate whether a quantitative model based on the concepts mentioned in this paper can reproduce some of the experimental findings on Na^+ replacement and on stimulation by carbachol or CCK. Furthermore, we will investigate what the model, once established, can tell us about the control and regulation of fluid secretion.

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