Increased phosphate efflux from acinar cell during protein secretion

TERI MELESE AND S. S. ROTHMAN
Laboratory of Cellular Dynamics, Departments of Physiology and Anatomy, School of Medicine, University of California, San Francisco, California 94143

MELESE, TERI, AND S. S. ROTHMAN. *Increased phosphate efflux from acinar cell during protein secretion.* Am. J. Physiol. 245 (Cell Physiol. 14): C121–C124, 1983.—The permeability of the pancreatic epithelium to two water soluble molecules, sucrose and inulin, increases when protein secretion is augmented by a cholinergic agonist. An increase in the permeability of passive paracellular shunts (3) has been proposed to account for these observations. In the present experiments we have measured the distribution of another molecule, phosphate ion, across the epithelium by following its secretion from the cannulated duct of whole-rabbit pancreas in short-term organ culture. A cholinergic stimulant increases phosphate ion concentration in secretion in a similar fashion to that seen for the watersoluble nonelectrolytes. However, both the unstimulated rate of phosphate secretion and the increase observed with cholinergic stimulation were not dependent on the presence of the ion in the medium, and therefore its secretion in both cases reflects phosphate efflux from the cell and not its paracellular transport. The results indicate that either the phosphate ion is excluded from paracellular shunts or that such shunts do not contribute substantially to the transpancreatic passage of molecules of this size.

pancreas; permeability; epithelial transport

A STIMULANT THAT AUGMENTS digestive enzyme secretion by the pancreas also causes an increase in the permeability of the gland to two nonelectrolytes, sucrose and inulin. Because these molecules are not thought to enter cells, their increased flux across the epithelium led to the proposal that the permeability change involved paracellular shunts (3). To consider this question for phosphate ion, we have measured its distribution across the epithelium before and after cholinergic stimulation of protein secretion, both when phosphate was present in the medium bathing a secreting pancreas in short-term organ culture and when it was absent. In its absence, the flux of phosphate into ductal fluid, and changes in that flux that might be seen with stimulation, can be attributed to cellular sources.

METHODS

Biological preparation. The pancreas was surgically removed from fasted (approx 18 h) male New Zealand White rabbits (2–3 kg body wt) under anesthesia produced by a mixture of allobarbital, urethan, and monoethylurea (0.8 ml/kg body wt, ip) (8). Before excision, the pancreatic duct was cannulated with polyethylene tubing (PE 10, Clay Adams, 28 cm in length). The pancreas was removed along with the surrounding loop of intestine and mounted on a frame as described elsewhere (6, 7). The mounted pancreas was then put into a Plexiglas chamber containing 400 ml of a Krebs-Henseleit solution (4) supplemented with glucose (5 mM) and amino acids (2) and either contained phosphate (1.2 mmol/l) or had phosphate omitted. The preparation was gassed with 95% O_2-5% CO_2, and its temperature was maintained at 30°C.

Experimental procedures. In all experiments the organ was preincubated for 30 min, the medium was replaced, and measurements were initiated. In one group of experiments $NaH_2{}^{32}PO_4$ (150 mCi/mmol, ICN) was added to a phosphate-containing medium when it was changed. In these experiments ductal fluid was collected in tared vials at two 5-min and then two 10-min intervals for the first 30 min, i.e., until the added label reached a steady-state concentration in ductal fluid, and then for two additional 15-min periods. At this time, methacholine chloride (1 mg/100 ml bath fluid) (MCh) was added and samples were collected for two 5- and two 10-min intervals in sequence for the next 30 min and then for two additional 15-min periods. In other experiments, where no radioisotope was added, the gland was preincubated for 30 min either in a medium containing phosphate (1.2 mmol/l) or in one containing no phosphate ion. After preincubation, the medium was replaced with new fluid of the same composition, two 15-min samples were collected, the stimulant (MCh) (1 mg/100 ml bath fluid) was added, and then four 15-min samples were collected. Removal of phosphate from the medium did not alter either fluid or protein secretion (see RESULTS) to any appreciable degree.

To determine radioactivity in ductal fluid, 25-μl aliquots were placed in scintillation vials with 1 ml of a dispersal agent (BBS-3, Beckman) to which approximately 19 ml of toluene containing fluors (TLA, Beckman) were added. The sample was then counted by liquid scintillation spectroscopy.

Phosphate was determined chemically by the technique of Fiske and SubbaRow (2) for total phosphate. Certain samples containing labeled phosphate were precipitated with 10% trichloroacetic acid, sedimented at 30,000 *g*, and the precipitate was washed three times. Aliquots of both supernatant and precipitate were

counted as described above. Protein in ductal fluid was estimated using the Lowry et al. (5) method, and a standard curve was prepared with bovine serum albumin.

RESULTS

^{32}P secretion in unstimulated and stimulated states. When ^{32}P was added to the medium it appeared in ductal fluid, reaching a steady-state concentration over a period of 20–30 min. Its concentration in the duct relative to that in the bath, expressed as percent of bath concentration, was approximately 8% (Fig. 1). When a cholinergic stimulant was added to the medium, ^{32}P concentration in ductal fluid increased, reaching a new steady state over a period of 60 min that was over four times the unstimulated value, now averaging 36% of bath concentration (Fig. 1)..

Proportion of inorganic to organic phosphate in ductal secretion. To determine whether phosphate in ductal secretion was associated with organic matter (essentially all of the organic material in pancreatic secretion is protein), samples of ^{32}P-labeled secretion taken from steady-state periods (both unstimulated and stimulated) (see Fig. 1) were precipitated with 10% trichloroacetic acid. Only a small percentage (3–6%) of the labeled phosphate was recovered in the precipitate, and over 90% was apparently inorganic phosphate for the periods of incubation examined (Table 1).

Phosphate output in the presence of phosphate in the medium. Phosphate output in ductal fluid in the unstimulated state was 16 nmol/15 min ± 4 (SE, $n = 5$) with phosphate present in the medium (Fig. 2). When the cholinergic stimulant (MCh) was added, phosphate output increased by almost three times, reaching an average value of 41 nmol/15 min ± 7 ($n = 6$ for 30 and 45 min and 5 for 60 min) between 30 and 60 min (Fig. 2). Phosphate concentration in ductal fluid, determined both chemically or isotopically, was approximately 40% of bath concentration under stimulated conditions (isotopically, 0.43 mM; chemically, 0.52 mM).

Phosphate output in the absence of phosphate in the medium. When phosphate was omitted from the medium, its output in the unstimulated state remained relatively unchanged; 13 nmol/15 min ± 1 (SE, $n = 6$) (Fig. 2). When MCh was added, phosphate output increased by nearly three times reaching a maximum value of 41 nmol/15 min ± 3 ($n = 6$) at 30 min (Fig. 2). Thus phosphate output and concentration in secretion was roughly the same whether or not phosphate was present in the medium in both unstimulated and stimulated cases (phosphate concentrations: unstimulated, 0.20 mM ± 0.06 with phosphate in medium and 0.17 mM ± 0.04 without; stimulated, 0.52 mM ± 0.02 with phosphate in medium and 0.48 mM ± 0.01 without).

Protein output in the presence and absence of phosphate. Protein output in ductal fluid was 0.37 mg/15 min ± 0.2 (SE, $n = 4$) in the unstimulated state when phosphate was in the medium (Fig. 3). When MCh was added, output reached a peak of 5.7 mg/15 min ± 0.3 ($n = 4$) at 30 min. By 60 min output had declined substantially to a value that was only 44% of the 30-min peak or 2.5 mg/15 min ± 0.4 ($n = 4$). In the absence of phosphate, protein output in the unstimulated state was about the same: 0.30 mg/15 min ± 0.1 ($n = 6$). When the stimulant

TABLE 1. *^{32}P (% of total) in ductal secretion at steady state*

	Unstimulated	Stimulated
Supernatant	97.0% ± 0.5	94.0% ± 0.7
TCA precipitate	3.0% ± 0.5	5.8% ± 0.7

Values are means ± SE. Samples are from 10 different unstimulated and 15 different stimulated periods in 3 rabbits.

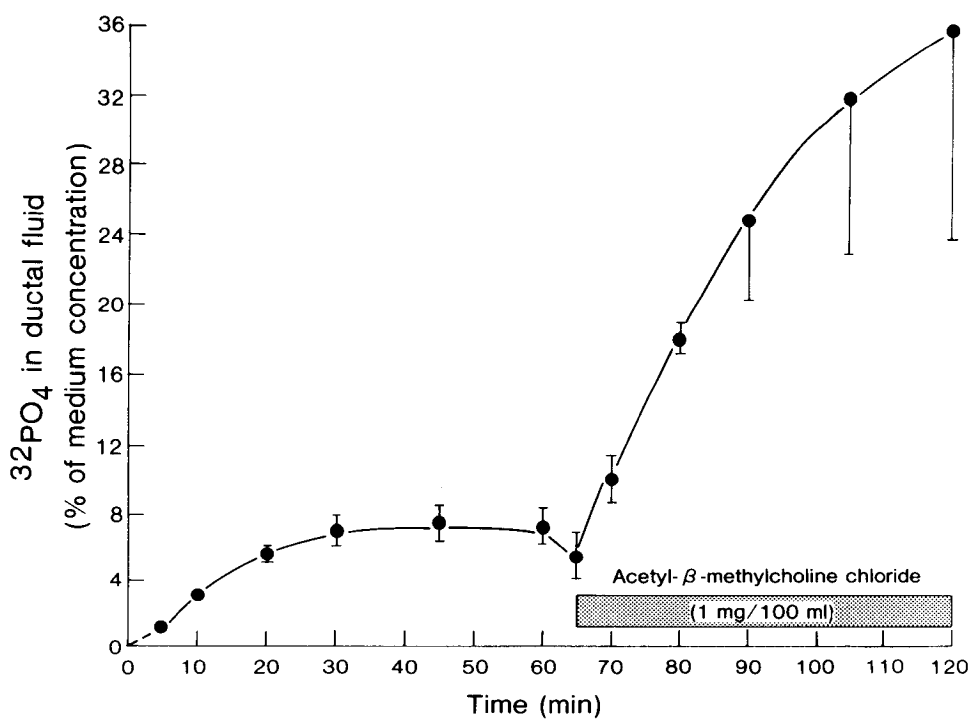

FIG. 1. Concentration of labeled phosphate in ductal fluid in unstimulated and stimulated states. Values are expressed as percent of bath concentration. The appearance of label was followed in samples of secretion collected from cannulated duct of rabbit pancreas in vitro in the continuous presence of label. After addition of a cholinergic stimulant (methacholine chloride; 1 mg/100 ml bath fluid) (MCh) (*bar*), concentration of labeled phosphate in ductal fluid increased to a new steady state approximately 4 times the unstimulated value. Values are means ± SE; $n = 3$. The absence of SE *bars* indicates that calculated value was within *point mark*.

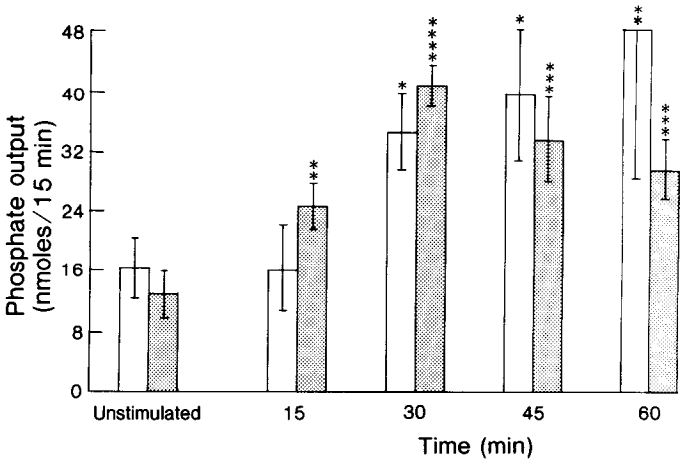

FIG. 2. Phosphate output in ductal fluid in unstimulated and stimulated states in the presence (*open bars*) and absence (*shaded bars*) of phosphate in the medium. Samples of secretion were collected from cannulated duct of the rabbit pancreas in vitro. Phosphate output, in both unstimulated and stimulated states, was approximately the same whether or not phosphate was present in the medium. "Unstimulated" values refer to 15-min period just prior to the addition of the stimulant. Values are means ± SE. −Phosphate, $n = 6$ for all periods; +phosphate, $n = 5$ for both control and 60-min periods, $n = 6$ for 15-, 30-, and 45-min periods. $^{*}P < 0.05$; $^{**}P < 0.025$; $^{***}P < 0.01$; and $^{****}P < 0.001$ compared with "unstimulated."

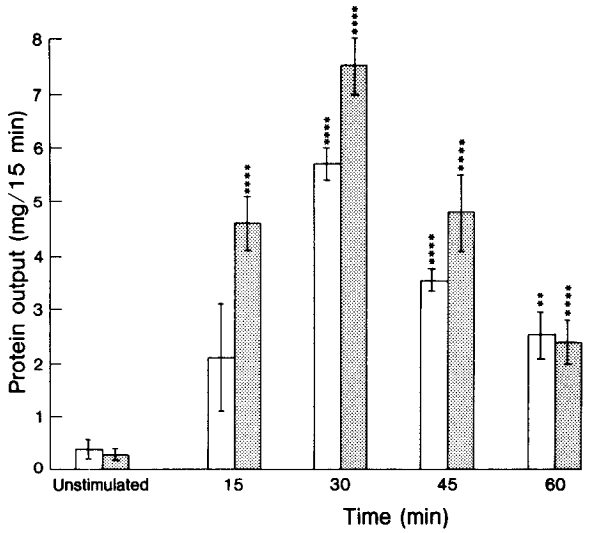

FIG. 3. Protein output in ductal fluid in unstimulated and stimulated states in the presence (*open bars*) and absence (*shaded bars*) of phosphate in the medium. Experimental system was as described in Fig. 2 and METHODS. Protein output increased after addition of stimulant, and response was roughly the same in terms of time course and magnitude whether or not phosphate was present in the medium. "Unstimulated" values refer to the 15-min period just prior to addition of stimulant. Values are means ± SE. +Phosphate, $n = 4$ except $n = 5$ at 45 min; −phosphate, $n = 6$ except $n = 4$ at 15 min. $^{**}P < 0.025$; $^{***}P < 0.001$ compared with "unstimulated."

was added, output again reached a peak at 30 min but at a somewhat higher value: 7.5 mg/15 min ± 0.8 ($n = 6$). Output fell over the next 30 min to 2.4 mg/15 min ± 0.4 ($n = 6$), or approximately 32% of the peak value (Fig. 3).

DISCUSSION

The concentration of phosphate ion in ductal fluid increased three- to fourfold with the addition of a cholinergic agonist that augments protein secretion. This behavior is similar to that previously reported for sucrose and inulin under the same circumstances in the same biological preparation (3).

The net flux of a molecule into the pancreatic duct system can potentially be the sum of two separate component fluxes; a paracellular and cellular flux. If a particular molecule is removed from the extracellular space then only the cellular flux can still occur. When phosphate ion was omitted from the medium bathing rabbit pancreas in short-term organ culture, no significant difference in the phosphate flux into ductal fluid was observed. Furthermore, stimulation of the gland under these circumstances led to an increase in phosphate efflux that was about the same as that seen when it was in the medium. Because no decrease in phosphate efflux was observed in its absence from the medium in either the unstimulated or stimulated state, then the paracellular component of the transepithelial phosphate flux must have either been small or nonexistent. Thus the increase in phosphate flux seen during augmented secretion either reflects an increase in cellular permeability to phosphate or an increase in the concentration gradient or driving force for this ion from cell to duct. On the basis of this evidence the only manner in which the flux of phosphate might nevertheless reflect its movement through a paracellular channel is if it were released solely across the basolateral surface of the secretory cell and if this efflux, in its entirety, traveled into the duct lumen rather than across the serosal surface of the tissue into the bathing medium. This could only occur if the reflexion coefficient of the shunt pathway was much lower than for its movement across the basal lamina. However, if this were so, then the total paracellular flux of phosphate into ductal fluid should have been greater when phosphate was present in the bathing medium at a concentration of 1.2 mM. This was not the case and phosphate concentration in ductal fluid was about 0.2 mM whether or not it was present in the medium. Thus it seems clear that the total transepithelial flux of phosphate is due to its transport across the secretory cell both in the unstimulated and stimulated state.

As we said earlier, the increase in phosphate flux that occurs coincident with augmented protein secretion is of the same general nature as that reported previously for nonelectrolytes, in terms of both its magnitude and time course. Either the phosphate response reported here and that seen for sucrose and inulin with stimulation (3) are different effects, one cellular and the other paracellular, or they are one and the same effect. If paracellular shunts in the pancreas can accomodate molecules the size of the phosphate ion or larger, then a substantial portion of the phosphate flux, certainly comparable to that reported for sucrose and inulin (3), would have been dependent on its presence in the medium. This was not the case, and transpancreatic phosphate passage could be wholly accounted for by its movement across cells. Thus either phosphate is excluded from the shunt, perhaps due to its charge, or shunts do not contribute substantially to the transpancreatic passage of molecules of this size.

The increased rate of efflux of molecules other than proteins from the secretory cell that occurs with aug-

mented protein secretion can be viewed in three ways: *1*) as tightly coupled to protein secretion, *2*) as a totally independent occurrence, and *3*) as related to protein secretion but not coupled to it. The present experiments do not explore these issues but they do give us some insight into them. In the presence of the stimulant the rate of protein secretion fell by one half to two thirds during a period of 30 min after reaching its peak value, whereas the increased flux of phosphate ion was maintained at a more or less constant level over this same time period and certainly did not decrease in parallel to protein secretion (Figs. 2 and 3). Thus if the two processes are related, the fluxes (protein and phosphate) are not tightly coupled. In other words, the increased efflux of phosphate at the apical membrane of the cell is not, at least in a quantitative fashion, dependent on the flux of proteins across this surface for its expression.

This work was supported by National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases Research Grant AM-16990.

Received 23 August 1982; accepted in final form 21 January 1983.

REFERENCES

1. EAGLE, H. Amino acid metabolism in mammalian cell cultures. *Science* 130: 432–437, 1959.
2. FISKE, C. H., AND Y. SUBBAROW. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375–400, 1925.
3. JANSEN, J. W. C. M., J. J. H. M. DEPONT, AND S. L. BONTING. Transepithelial permeability in the rabbit pancreas. *Biochim. Biophys. Acta* 551: 95–108, 1979.
4. KREBS, H. A., AND K. HENSELEIT. Untersuchungen über die Harnstoffbildung im Tierkörper. *Z. Physiol. Chem.* 210: 33–66, 1932.
5. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275, 1951.
6. ROTHMAN, S. S. Exocrine secretion from the isolated rabbit pancreas. *Nature London* 204: 84–85, 1964.
7. ROTHMAN, S. S., AND F. P. BROOKS. Electrolyte secretion from rabbit pancreas in vitro. *Am. J. Physiol.* 208: 1171–1176, 1965.
8. ROTHMAN, S. S., AND H. WELLS. Selective effects of dietary egg white trypsin inhibitor on pancreatic enzyme secretion. *Am. J. Physiol.* 216: 504–507, 1969.